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Volume VII 1915–16

Cambridge University Press
C. F. CLAY, Manager
LONDON: Fetter Lane, E.C.
EDINBURGH: 100, Princes Street
also H. K. LEWIS & Co., Ltd., 136, Gower Street, London, W.C.
and WILLIAM WESLEY & SON, 28, Essex Street, London, W.C.
CHICAGO: The University of Chicago Press
BOMBAY, CALCUTTA AND MADRAS: Macmillan and Co., Ltd.
TORONTO: J. M. Dent and Sons, Ltd.
TOKYO: The Maruzen-Kabushiki-Kaisha

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Cambridge:
PRINTED BY JOHN CLAY, M.A.
AT THE UNIVERSITY PRESS.

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THE ATMOSPHERE OF THE SOIL: ITS COMPOSITION AND THE CAUSES OF VARIATION.

BY EDWARD JOHN RUSSELL AND ALFRED APPLEYARD.

(*Rothamsted Experimental Station.*)

(With 17 Text-figures.]

Introduction.

THE remarkable relationships existing between the microorganisms of the soil and the growth of plants have given rise to numerous researches on the bacteria, fungi, and more recently the protozoa of the soil, and considerable knowledge has now been obtained of the organisms present in normal soils. The earlier investigations were necessarily confined largely to methods of isolation, descriptions of the organisms found and studies of their behaviour in certain culture solutions, but sufficient of this preliminary work has been done to enable us to attack the real problem and try to obtain a picture of the life in the soil as it actually is. For this purpose it is necessary to know the relative abundance of the various organisms, to find out which are active and which inert, and to discover what the active forms are doing and what is their mode of life. Before the bacteriological and zoological work can be fully interpreted, however, it is necessary to discover the conditions under which life in the soil goes on, and in the series of papers, of which this is the first, it is proposed to deal with the air supply, water supply, and temperature of our own soils and by comparison with other investigations to see how far the observed relationships hold generally.

In the present paper we shall confine ourselves to the atmosphere of the soil. The soil mass is porous and the volume of solid matter in our case[1] is approximately two-thirds of the whole, leaving one-third pore space. The pore space, however, is not empty but contains a considerable amount of water, and the actual space empty except for air is commonly not more than 10 to 20 per cent. of the volume of the soil. The pores appear to be continuous and seem to maintain fairly complete communication between the various layers of the soil; in some places the communication is made more effective by the presence of cracks and burrows.

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[1] For analysis of the soil, see p. 44.

The soil atmosphere is the air present in these pores. Its biological significance lies in the fact that it is the air surrounding the soil organisms and the roots of plants, and is either in actual contact with them or is separated from them only by a thin film of water or colloidal matter. It is obviously part of the ordinary atmosphere but its composition is influenced by two causes: oxygen is absorbed and carbon dioxide produced by the inhabitants of the soil; while on the other hand, diffusion and other processes of gaseous interchange are constantly replacing it with ordinary atmospheric air, thus eliminating any differences in composition brought about by biochemical or other changes. As a net result the composition of the soil air at any moment is determined by the difference of velocity with which these two processes take place.

Unfortunately the mechanism of gaseous interchange in the soil is not sufficiently well known to enable us to ascertain the speed at which it goes on and so to discover the rate of production of carbon dioxide, a quantity of great importance in the study of the biochemical changes in the soil, but we have obtained evidence that our curves are mainly determined by the production and not by the loss of carbon dioxide from the soil. In any case for our present purpose of discovering the conditions under which life goes on in the soil it is mainly necessary to know the resultant of the various actions concerned.

Preliminary determinations showed that it is not difficult to draw a sample of gas from the soil, that is fairly representative of the soil air and is uncontaminated by atmospheric air In our experiments the depth selected has been 6 inches, this being right in the region where the soil changes take place, besides being convenient for working. But as a matter of fact no great difference in composition was found on going somewhat deeper: thus the following results (Table I) were obtained at 6 and 18 inches respectively.

In general the soil air was found to be very similar in composition to ordinary atmospheric air, especially as regards the percentages of oxygen and of nitrogen. It commonly contains less oxygen and more carbon dioxide, usually also more nitrogen, but the differences are often small and only detected with certainty by careful analyses (Table VI).

TABLE I. *Comparison of composition of soil air taken from a depth of 6 and 18 in. in the soil. 30 January 1914. Percentage by volume.*

	CO_2		O_2	
	6″ deep	18″ deep	6″ deep	18″ deep
Grassland, Greatfield	1·46	1·64	18·44	17·87
Arable land, Broadbalk (dunged plot) ..	0·34	0·50	20·52	20·33
Arable land, Broadbalk (unmanured plot)	0·34	0·45	20·32	20·35

Unlike atmospheric air, however, the soil air is not constant in composition but changes somewhat from day to day and even on the same day at different spots in the field; nevertheless the values fall within fairly narrow limits.

There are two kinds of variation in composition; the local daily ones just referred to, and the greater variations produced by season, treatment, etc.: the latter may be so great as to mask altogether the local fluctuations. In our experiments the greatest factor of all was the effect of season. Whatever the history of the soil its atmosphere in spring and to a less extent in autumn was characterised by high amounts of carbon dioxide indicating rapid biochemical changes at these seasons of the year, while in summer and winter the amounts were much lower. The effect is complex and includes at least two others each of which was found to be very potent: the temperature during the period December to June, and moistness of the soil during part of the summer months. (Figs. 7 and 8.)

In addition there is the possibility that a certain amount of partial sterilisation has taken place during the winter and during the dry summer, leading to considerable bacterial activity immediately conditions become favourable once more.

This seasonal effect dominates all the others and impresses on all the curves the same general type seen in Figs. 1–6[1]. Other factors, such as manuring, cropping, etc., simply raise or lower the whole curve according as they give rise to more or less carbon dioxide; in particular the effect of the crop proved to be considerably less than was anticipated.

Within these major variations there fall the smaller fluctuations attributable to differences in composition of the soil[1], especially the distribution of organic matter, organisms, plant roots and passages such as cracks, burrows of earthworms, etc.; to daily changes in temperature and moisture content of the soil, or to any cause that would facilitate interchange between the soil air and the atmosphere. These local and daily fluctuations lie between relatively narrow limits, and by taking a mean of a number of samples it is not difficult to arrive at a value that approximately expresses the composition of the soil air at the time. Some of these values are given in Table II.

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[1] See Table VI for data.

TABLE II. *Mean composition of soil air from various Rothamsted plots. Percentage by volume.*

	CO_2	O_2	N_2
Arable land manured (farmyard manure) and cropped			
Broadbalk wheat Plot 2 — Summer	0·23	20·74	79·03
Winter	0·37	20·31	79·32
Arable land unmanured and cropped			
Broadbalk wheat Plot 3 — Summer	0·19	20·82	78·99
Winter	0·21	20·42	79·37
Arable land unmanured and cropped Hoos wheat			
Summer	0·28	20·65	79·07
Winter	0·20	20·71	79·09
Arable land unmanured and cropped Hoos fallow			
Summer	0·12	20·84	79·04
Winter	0·08	20·78	79·14
Mean of all the arable soils .. .	0·25 ± 0·1	20·6 ± 0·2	79·2 ± 0·2
Pasture land. Winter	1·57	18·02	80·04
Atmospheric air	0·03	20·97	79·00

The column labelled nitrogen is simply the residual gas after the carbon dioxide and oxygen have been removed in the analytical process and it includes other gases just as in the case of atmospheric air. Sir James Dewar kindly examined some of the samples for hydrogen, but found only quantities of the same order as in the atmosphere, while our own tests have failed to reveal appreciable quantities either of methane or any other combustible gas. We may therefore safely assume that the residual gas is practically all nitrogen.

---

[1] We are here using the word to denote the whole of the surface soil complex: solid matter, water, air spaces, etc. It is unfortunate that no soil chemist has yet had the courage to coin a word to express this meaning. The word "soil" is ambiguous, as it means also the actual solid matter.

This then represents the ordinary composition of the air filling the pores of the soil at a depth of 6 inches, the layer within which most of the important soil changes go on. As already pointed out it is very similar to ordinary atmospheric air but there are three important differences which may have much greater effects than would at first be expected:

1. The amount of carbon dioxide though low in the absolute, is nevertheless about ten or more times as high as in atmospheric air.

2. The amount of moisture present in the soil air is greater than in atmospheric air and is usually nearer the saturation point.

3. The soil air is still, there being much less opportunity for actual movement than in the atmosphere.

It is outside our present subject to discuss the effects of these characteristics and we need only indicate a few ways in which they may be expected to act.

There is considerable evidence that microorganisms are very sensitive to the medium in which they are placed, and the relatively high proportion of carbon dioxide in the soil atmosphere is likely to affect their activity. It is therefore necessary to take this factor into account before applying to the soil any deductions from bacteriological investigations made in the laboratory under ordinary atmospheric conditions.

In consequence of its stillness and its intimate contact with the moist soil particles, the soil air is likely to be saturated or nearly saturated with water vapour, and this condition is known to be favourable for organisms and to reduce the need for free liquid water.

The effect of the extreme stillness of the air, however, cannot be gauged; physiologists recognise that movement in the air is necessary for the comfort and well being of humans, and we should no doubt find the soil atmosphere intolerable from this cause alone, but it is difficult to form any estimate of its effect on microorganisms.

But this free air filling the pore spaces is not the only air in the soil. During the course of other experiments we had occasion to evacuate flasks containing soil, and we found that the vacuum persistently began to fall soon after exhaustion appeared to be complete. Gas was being evolved from the soil, but it came out only very slowly even when a good mercury pump was kept at work for several days.

The total amount of gas given up is not great; its characteristic feature is the absence of oxygen (except in small quantities) and the high proportion of carbon dioxide.

Some of the samples obtained had the composition shown in Table III.

TABLE III. *Composition of gas held absorbed by soil. Percentage by volume.*

	Weight of soil used, grms	Percentage of Moisture	Approximate volume of gas removed in successive extractions	Percentage composition of gas		
				CO_2	O_2	N_2
Pasture soil	352	28	1st 30 c.c.	52·0	0·7	47·3
			2nd 30	84·8	0·2	15·0
			3rd 22	99·1	0·2	0·7
Soil covered with vegetation (Broadbalk wilderness)	400	22	1st 30 c.c	19·3	5·5	75·2
			2nd 30	57·0	2·6	40·4
			3rd 15	98·7	0·2	1·1
Rich garden soil	468	20	1st 30 c.c.	89·5	0·2	10·3
			2nd 30	99·3	0·0	0·7
			3rd 15	94·4	0·0	0·6
	extracted later		4th 30	96·8	0·0	3·1
			5th 30	92·3	0·0	7·6
Arable soil Broadbalk dunged plot .	—	24	1st 30 c.c.	10·8	4·4	84·8
			2nd 30	57·9	1·8	40·3
			3rd 15	98·4	0·0	1·6
Broadbalk unmanured ..	497	16	1st 30 c.c.	6·3	15·1	78·6
			2nd 25	40·2	9·7	50·1

It will be observed that the composition varies with the pressure, and that the first samples withdrawn contain more oxygen than the last: the final samples are almost pure carbon dioxide.

The volume of gas obtainable depends on the amount of moisture in the soil as it is brought in from the field, and decreased as the soil becomes dryer; from which we may infer that the gas is partly dissolved in the soil moisture, though part may be dissolved in other soil constituents.

Thus it appears that there are two atmospheres in the soil: one present as free gas filling the pores, and practically as rich in oxygen as ordinary air, the other dissolved in the surface films of water and other substances, almost devoid of oxygen and consisting mainly of carbon dioxide with some nitrogen.

It is hardly likely on physical grounds that these atmospheres are abruptly parted at the surface of the film; it is more probable that the free air changes in composition at the surface of the particles where a thin layer of it is to some degree in equilibrium with the dissolved air. The stillness of the soil air is favourable to the formation of a stratum different in composition from the bulk and merging insensibly into it.

The very small amount of oxygen in the dissolved gas is evidence that the rate of consumption of oxygen in the solution is greater than the rate at which fresh supplies come in from the soil air, a fact of great biochemical significance. But still more important for our present purpose is the fact of the existence of this atmosphere almost devoid of oxygen.

We are accustomed to think of a drained cultivated soil as being under essentially aerobic conditions, and the analyses of the free air show that this view is correct. But the existence of this second atmosphere enables an organism that wants anaerobic conditions to find them by submerging itself into the medium in which this atmosphere is dissolved, especially if at the same time it associates itself with an aerobic form capable of taking up any oxygen that becomes dissolved. Thus alongside of the aerobic life in the soil there is the possibility of anaerobic life, and we can no longer dismiss a possible soil change as unlikely simply on the grounds that it requires anaerobic conditions. In the present paper we confine ourselves to the free air in the soil but hope to deal with the dissolved air later on.

The free air in the soil.

For the first examination of the free air of the soil we have to turn, as in many other agricultural studies, to the papers of Boussingault. In 1853 he published[1] the results of analyses of 36 samples of soil gas taken at a depth of 30–40 cms. At that time Bunsen's classical memoir had not been published nor had gas analysis methods been worked out, so that he was compelled to fix a pipe in the soil (thus causing considerable disturbance) and periodically to aspirate a large volume (2½ to 10 litres) of soil air through baryta water and weigh the carbonate formed. The method must have been cumbersome to work; nevertheless the results are fairly close to ours, the air obtained from soils that had not recently been manured having the following mean composition:

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[1] Boussingault and Léwy, 'Mémoire sur la composition de l'air confiné dans la terre végétale,' *Annales de Chimie et de Physique*, 1853, **37**, 5–50.

Carbon dioxide	0·9	per cent. by volume
Oxygen	19·6	„ „ „
Nitrogen	79·5	„ „ „

It is clear that the method gives rather high results for carbon dioxide because atmospheric air was found to contain 0·04 per cent. instead of 0·03 per cent. The air from a recently manured soil contained much more carbon dioxide—up to 10 per cent.—while the oxygen fell as low as 10 per cent.[1]: but as these are the only two out of the 36 they have been omitted from the general mean.

Boussingault and Léwy did not continue their analyses over any prolonged period, nor did they study the effect of conditions such as temperature, moisture content, etc., on the composition of the soil atmosphere. These problems were investigated in Germany and the work was the outcome of the discovery by Pettenkofer[2] of a simple and rapid method of estimating carbon dioxide which he successfully applied in determining the amount of carbon dioxide in the air of the Munich soils[3]. This new method was much more rapid than the older one of Boussingault, enabling many determinations to be made and not requiring great skill in manipulation. Hence a number of workers took it up and a succession of papers on the subject appeared in Wollny's Journal[4] also published from Munich.

It is unnecessary to review all the papers in detail: especially as this has already been done by Fodor[5], Wollny[6], and Letts and Blake[7]. Moreover, later work has shown that the results are about 30 per cent. too high[8]. For comparative purposes, however, the method serves

[1] We cannot help thinking there must have been some mistake here; in our experience the oxygen falls very low only in waterlogged soils (p. 32).

[2] Letts and Blake (*Proc. Roy. Soc. Dublin*, 1900, **9**, 116) have shown that the principle of the method had already been used by Dalton and his pupils, but this work seems to have been unknown to Pettenkofer.

[3] M. von Pettenkofer, 'Ueber den Kohlensäuregehalt der Grundluft im Geröllboden von München in verschiedenen Tiefen und zu verschiedenen Zeiten,' *Zeitsch. f. Biologie*, 1871, **7**, 395–417; and 1873, **9**, 250–257.

[4] *Forschungen auf dem Gebiete der Agrikultur-Physik*, 1878–1898.

[5] J. Fodor, *Hygienische Untersuchungen über Luft, Boden und Wasser*, Braunschweig, 1881.

[6] E. Wollny, *Die Zersetzung der organischen Stoffe*, 1897.

[7] E. A. Letts and R. F. Blake, 'The carbonic anhydride of the atmosphere,' *Proc. Roy. Soc. Dublin* 1900, **9**, 107–270, especially pp. 214 *et seq.*

[8] Caldwell, in Letts and Blake's paper, *Proc. Roy. Soc. Dublin* 1900, **9**, 219–229.

sufficiently well. Successive workers showed that the amount of carbon dioxide in the soil air increased with the amount of organic matter, the water content, and the temperature of the soil. On one point, however, there was considerable disagreement which has survived to our own day: the effect of a growing crop on the production of carbon dioxide in the soil. F. Ebermayer[1] found less carbon dioxide in the soil of a wood than in a fallow soil. Möller[2] in one experiment found more carbon dioxide when a crop of grass was growing, in another less, but the conditions were not strictly comparable. In a better experiment Wollny[3] found that the effect depended on the season: in summer the cropped land (grass) was poorer in carbon dioxide than the fallow land while in winter it was richer. Of the various papers published during this early period this one by Wollny is of rather special interest because it contains numerous CO_2 values obtained between May and September which show an early summer minimum and late summer (end of August) maximum just like ours do. Numerous determinations were also made by Fodor at depths of 1, 2 and 4 metres below the surface of the soil and these showed a maximum percentage of CO_2 in July and a minimum in January or March[4]. No spring maximum was observed.

The earlier workers ascribed the formation of carbon dioxide to the decomposition of the organic matter and generally assumed that the process was the purely chemical "eremacausis" pictured by Liebig. But it was gradually recognised that soil contained numbers of microorganisms and in 1880 Wollny[5] adopting the method of Schloesing and

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[1] Ebermayer, 'Mitteilungen über den Kohlensauregehalt der Waldluft und des Waldbodens im Vergleich zu einer nicht bewaldeten Fläche,' *Forsch. auf dem Gebiete der Agrik.-Physik*, 1878, **1**, 158–161.

[2] Joseph Möller, 'Ueber die freie Kohlensäure im Boden,' *ibid.* 1879, **2**, 329–338.

[3] E. Wollny, 'Untersuchungen über den Einfluss der Pflanzendecke und der Beschattung auf dem Kohlensäuregehalt der Bodenluft,' *ibid.* 1880, **3**, 1–15.

[4] Fodor, *loc. cit.* pp. 125 *et seq.*

[5] 'Untersuchungen über den Kohlensäuregehalt der Bodenluft,' *Landw. Versuchs. Stat.* 1880, **25**, 373–391.

An earlier reference to the possible significance of microorganisms in producing the carbon dioxide of the soil occurs in a paper by Joseph Möller, 'Ueber die freie Kohlensäure im Boden' (*Mitt. aus dem forstlichen Versuchswesen Oesterreichs*, 1878, Heft. 2, 121–148). After showing that the amount of carbon dioxide is increased by additions of organic matter he goes on to state that the lower organisms and organic residues brought in from the air are of considerable importance in this connection.

We have been unable to see the original paper, but in the long abstract in Wollny's *Forschungen* no reference is made to any experiments and it does not appear that this was more than an expression of opinion. At any rate it made no impression and it is not referred to by other writers, nor even by Möller himself in his second paper already quoted.

Müntz demonstrated that these were the active agents, the proof being that, in presence of chloroform, soil produces only a fraction of the amount of carbon dioxide formed in untreated soil. This was confirmed by Déherain and Demoussy[1]. From that time it has been generally recognised that the carbon dioxide is mainly produced by the organisms of the soil.

The application of the Pettenkofer method had thus carried the problem a long way, and had given considerable information about the origin and fluctuations of the carbon dioxide in the soil air, but it gave no information at all about the oxygen, and the idea gradually became fixed that the soil atmosphere was deficient in oxygen, a view that was strengthened by the well-known benefits of "aerating" the soil.

Boussingault and Léwy had indeed shown that the percentage of oxygen in the soil air was almost the same as that in the atmosphere, but their results were overlooked. As a matter of fact they rather contributed to the growth of the idea, for in their paper they laid chief stress on the fact that soil air contained 22 times as much carbon dioxide as ordinary air, and did not emphasise its close similarity in oxygen content.

With the introduction of improved methods of gas analysis it became possible to obtain still further refinements in the study of the soil atmosphere. Schloesing *fils*[2] was one of the first to apply the new methods and although his investigation was not very extensive it sufficed to demonstrate the incorrectness of the current conception that the soil air was necessarily deficient in oxygen.

In 1880 Hempel published his book describing a fairly accurate form of gas analysis apparatus which is as easy to use as Pettenkofer's and readily allows of the examination of large numbers of samples of air taken from the soil. It was adopted by Erich Lau in a series of analyses of the air from the soil at Rostock[3], one sample a month being taken from a sand, a loam, and a peat soil. The general result is that the soil air closely resembles ordinary air in its oxygen content, but that it contains about six times as much carbon dioxide; the actual mean values obtained at a depth of 15 cm. were, in percentages by volume:

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[1] *Ann. Agron.* **22**, 305.

[2] Th. Schloesing *fils*, 'Sur l'atmosphère confinée dans le sol,' *Compt. Rend.* 1889, **109**, 618–20, 673–76.

[3] Erich Lau, *Beiträge zur Kenntnis der Zusammensetzung der im Ackerboden befindlichen Luft*, Inaug. Dissertation, Rostock, 1906.

	Sand	Loam	Peat	Sandy soil, dunged	
				Cropped with potatoes	Fallow
Carbon dioxide	0·11	0·14	0·43	0·57	0·18
Oxygen	20·79	20·69	20·35	20·22	20·73
Nitrogen	79·10	79·17	79·22	79·21	79·29

The minimum amounts of carbon dioxide (0·04, 0·05 and 0·12 per cent. in the sand, loam, and peat respectively) were found in February, the maximum (0·18, 0·31, and 0·81 per cent.) in July and August: no spring maximum was observed, but this might easily have been missed in the five weeks that elapsed between the taking of the May and the June samples. Some of the plots were planted and some not: the former contained more carbon dioxide than the latter, even in the summer; a result directly opposite to that obtained by Wollny.

Jodidi and Wells adopted Orsat's simpler form of the apparatus, and made a great number of analyses of the soil air from certain plots at Ames, Iowa, over the period April to August, 1910. The mean of all the results showed that at a depth of 7 inches the percentage of oxygen is 20·51, of carbon dioxide 0·25, and of nitrogen 79·24.

These various results are set out in Table IV and taken in conjunction with our own (Table VI) they establish beyond any reasonable doubt the close similarity between the soil air and the atmospheric air so far as oxygen and nitrogen content are concerned.

TABLE IV. *Mean composition of soil air.*

Percentage by volume of:			Locality	Investigators	Date
Oxygen	Nitrogen	Carbon dioxide			
20·6±0·2	79·2±0·2	0·2±0·1	Rostock, Germany	Erich Lau	1906
20·4±0·2	79·4±0·2	0·2±0·2	Ames, Iowa	Jodidi and Wells	1911
20·6±0·2	79·2±0·2	0·25±0·1	Rothamsted	Appleyard and Russell	1913–14

These figures are the means of the averages of the various plots.

The significance of the fluctuations in composition in the soil air.

As already stated the composition of the soil air at any moment is a resultant effect, being the difference between the rate at which the carbon dioxide is produced in the soil and that at which it is lost. At first sight it might appear that the composition must therefore be largely accidental but we have been able to show that it is not, and that the great fluctuations as distinct from the minor ones (p. 33) are regulated mainly by the rate of production of carbon dioxide in the soil. The method consists in finding some other substance in the soil which is *produced* in the same manner as the CO_2, but *lost* in a different way. If the curve showing the fluctuations of this substance is like the curve for CO_2 it follows that the fluctuations are largely governed by the rate of production and therefore that the curves given in Figs. 1–5 are essentially production curves. If on the other hand the fluctuations do not resemble those of CO_2 it follows that the curves are not essentially production curves but that their shape is due to a fortuitous balance of losses and gains.

The required substance is found in the nitrates of the soil which, like the carbon dioxide, are produced in the decomposition of the soil organic matter by bacteria but which are lost in a wholly different manner. Carbon dioxide is lost by gaseous diffusion, a process which proceeds most rapidly in dry conditions when the pores of the soil are most widely open: and least rapidly in wet conditions when the pores are more or less closed. The nitrates, on the other hand, suffer least loss under dry conditions and most loss in wet weather.

Determinations were therefore made of the amount of nitrate present in each plot on every occasion when samples of gas were drawn for analysis, and the values are plotted in the curves: unfortunately the necessity for this was not seen when the investigation first began so that no values were obtained during the first four months.

Inspection of the curves shows that they are all of the same type: there is some displacement in point of time but no difference in character. It follows then that the character of the fluctuations of CO_2 content in the soil air is determined by the rate of biochemical change in the soil. Further proof is afforded by the fact that the curves for bacterial numbers also show a close resemblance to those of CO_2 in the soil air.

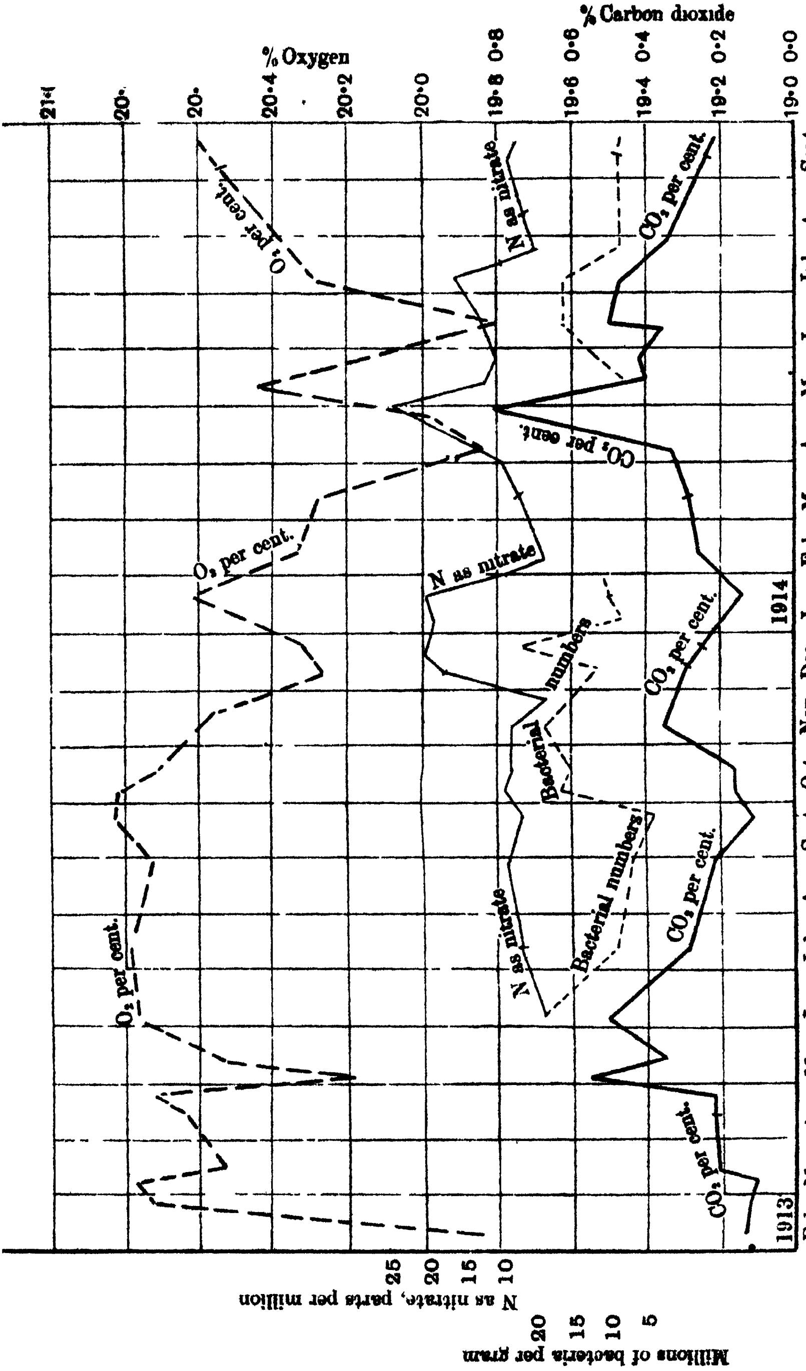


Fig 1 Curves showing percentage of CO_2 and O_2 in soil air and bacterial numbers (millions per gram). and nitrate (parts per million) in Broadbalk unmanured plot.

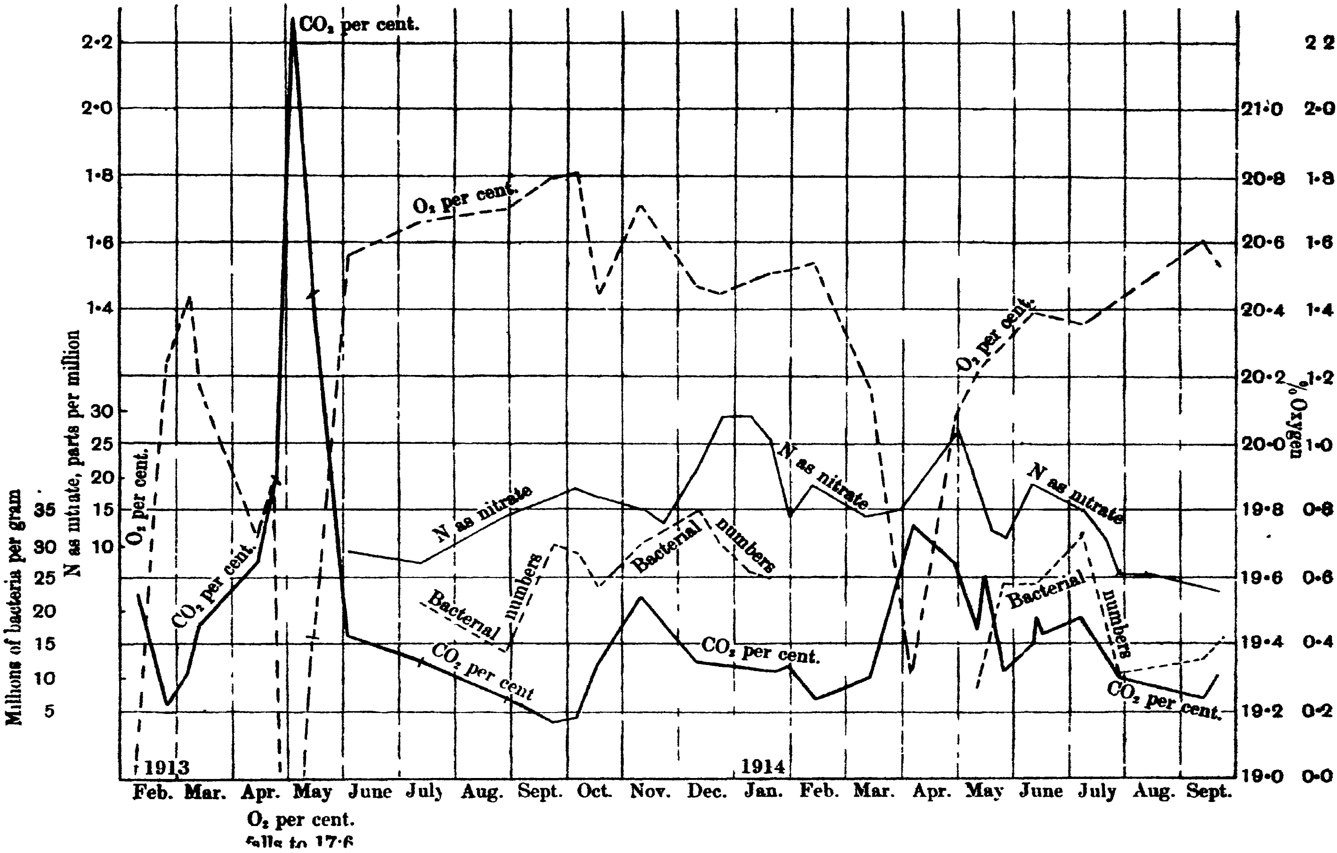


Fig. 2. Curves showing percentage of CO_2 and of O_2 in soil air, and bacterial numbers (millions per gram) and nitrate (parts per million) in Broadbalk dunged plot.

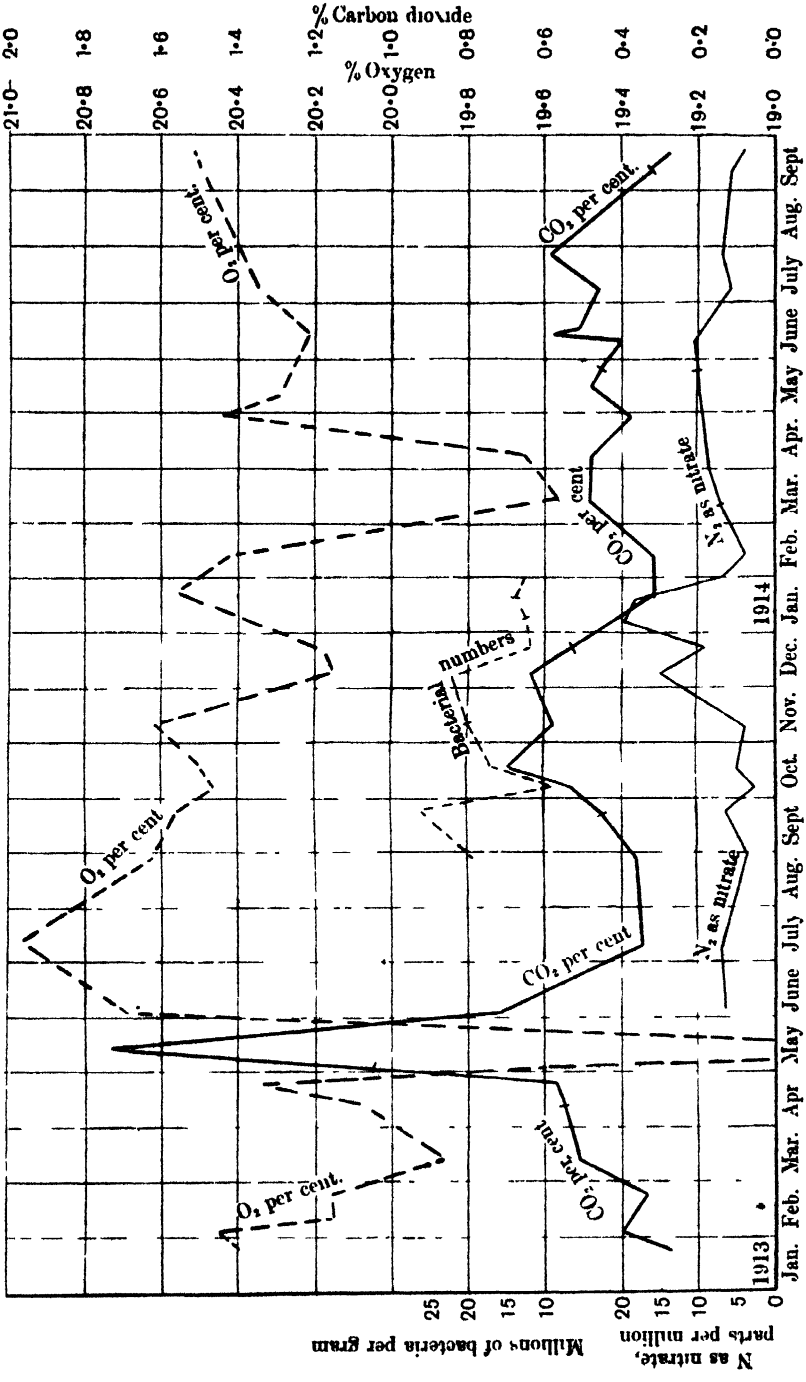


Fig. 3. Curves showing percentages of CO_2 and of O_2 in soil air, and bacterial numbers (millions per gram) and nitrate (parts per million) in Broadbalk wilderness

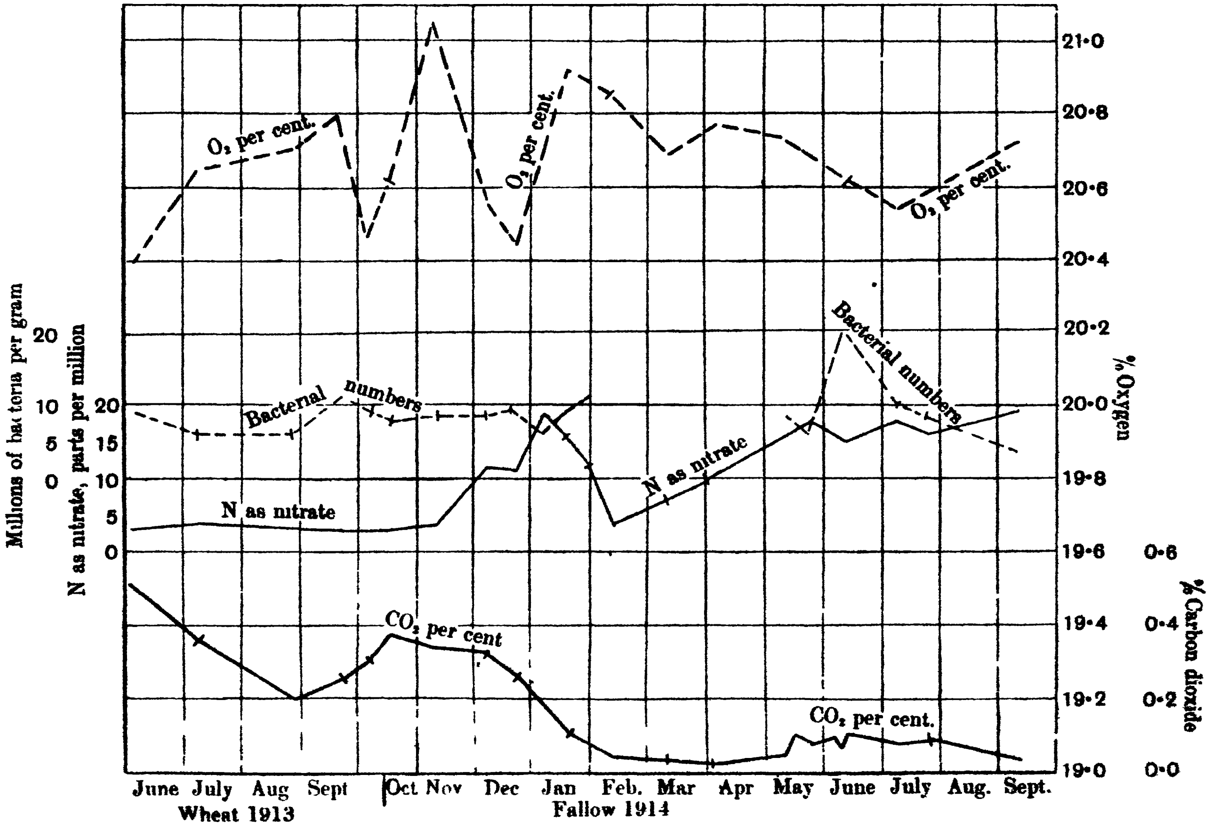
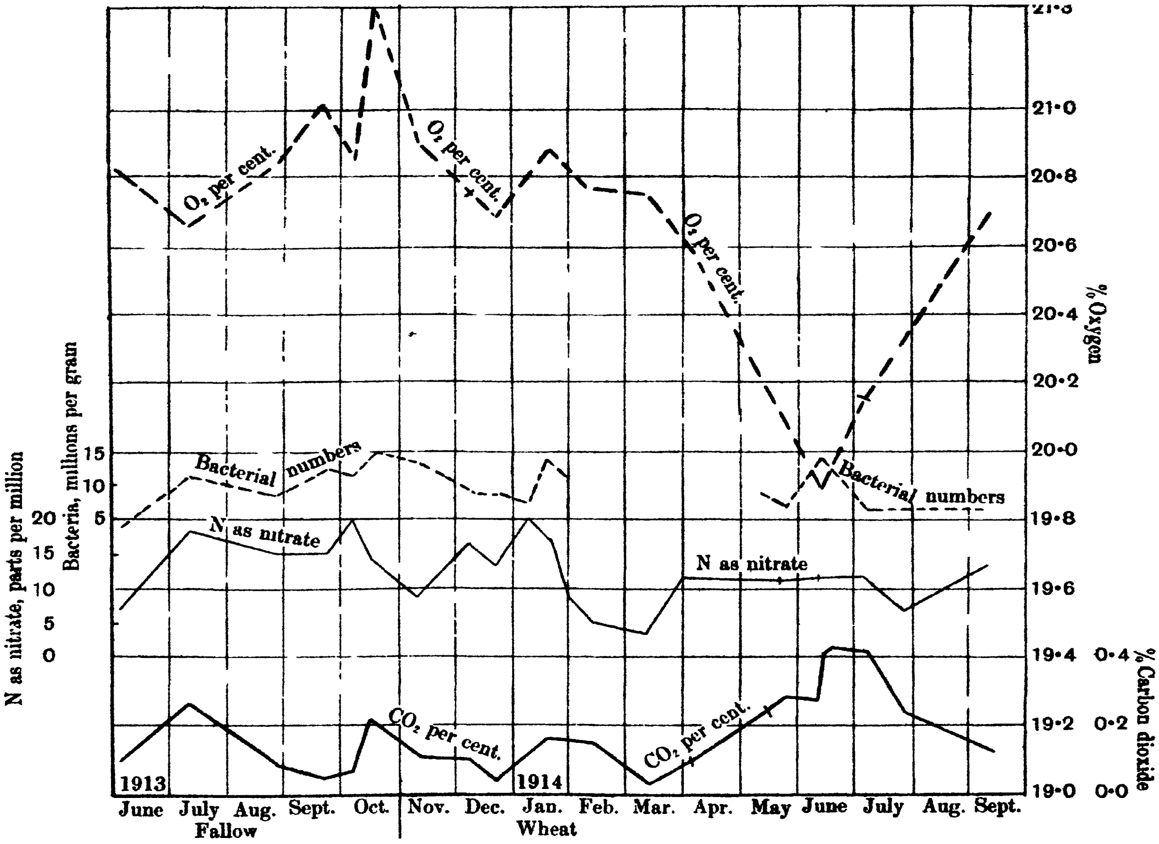


Fig 4 Curves showing percentages of CO_2 and O_2 in soil air of Hoos wheat and fallow plots.
(*a*) Cropped till Sept 1913, Fallow during season 1913–14.



ig 5. Curves showing percentages of CO_2 and of O_2 in soil air of Hoos wheat and fallow plots.
(*b*) Fallow till Oct. 1913, cropped during season 1913–14.

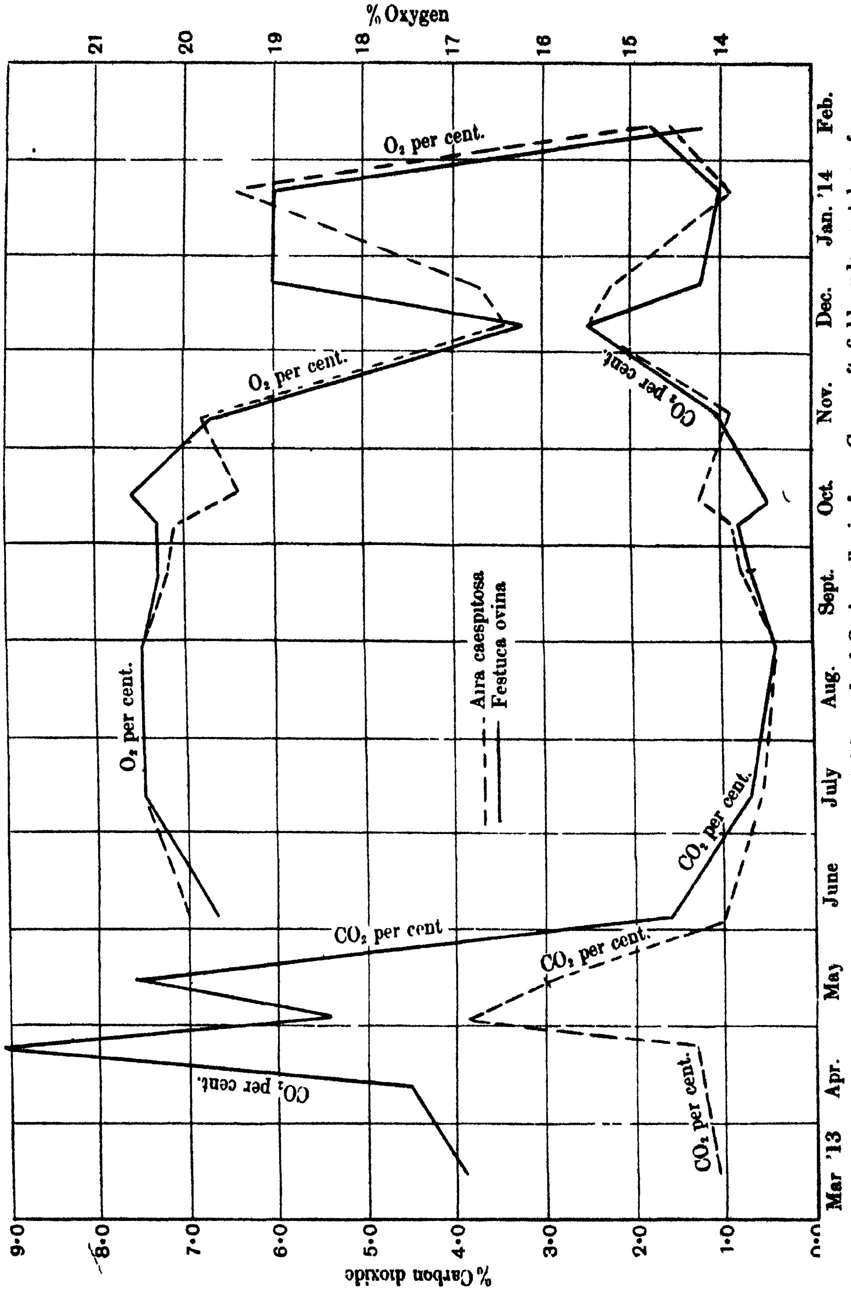


Fig. 6. Curves showing percentages of CO_2 and of O_2 in soil air from Geescroft field under patches of *Aira caespitosa* and of *Festuca ovina* respectively.

The relationship of CO_2 to oxygen.

The oxygen curves are generally reciprocal to the CO_2 curves, *i.e.* the oxygen falls as the CO_2 rises, and the agreement is sufficiently close to justify the assumption that the oxygen is mainly used up in producing CO_2. But the agreement is not absolute and the discrepancies are considerably beyond the limits of experimental error.

TABLE V. *Relationship of CO_2 to oxygen at times of rapid nitrification.*

Plot	Period	CO_2 in soil air %	O_2 in soil air %	Sum	Fall in O_2 in excess of rise in CO_2	Increase in nitrate during period, parts of N per million
Broadbalk dunged	Nov. 1913	0·54	20·72	21·26		
	Dec. ,,	0·35	20·47	20·82	0·44	7
	Mar. 1914	0·30	20·16	20·46		
	April ,,	0·76	19·31	20·07	0·39	13
	May ,,	0·44	20·22	20·66		
	June ,,	0·43	20·39	20·82	−0·16	7
Broadbalk wilderness	Nov. 1913	0·58	20·62	21·20		
	Dec. ,,	0·64	20·17	20·81	0·39	11
	Dec. ,,	0·53	20·19	20·72		
	Jan. 1914	0·32	20·55	20·87	−0·15	9
Broadbalk unmanured	Nov. 1913	0·35	20·56	20·91		
	Dec. ,,	0·29	20·27	20·56	0·35	10
	Mar. 1914	0·29	20·28	20·57		
	April ,,	0·34	19·85	20·19	0·38	17
Hoos fallow	Nov. 1913	0·33	21·06	21·39		
	Dec. ,,	0·32	20·57	20·89	0·50	8
	Feb. 1914	0·04	20·85	20·89		
	May ,,	0·05	20·73	20·78	0·11	11
Hoos wheat	Nov. 1913	0·11	20·90	21·01		
	Dec. ,,	0·10	20·76	20·86	0·15	8
	Mar. 1914	0·03	20·75	20·78		
	April ,,	0·10	20·58	20·68	0·10	7

At least two cases occur in which the oxygen decreases to a greater extent than the CO_2 increases:

(1) At times of active nitrification.
(2) After heavy rainfall.

In the first case the falling off of oxygen is partly at any rate the result of oxidations such as the production of nitrate which do not yield a volume of CO_2 equal to that of the oxygen absorbed. Table V gives the results obtained for all the periods of rapid nitrate accumulation: in all except two the fall in oxygen is greater than the rise of CO_2.

The second case is seen in wet weather particularly in February, 1913 and 1914, but it reaches its maximum development on Geescroft during the period when the soil lies waterlogged; the oxygen then falls as low as 2·6 per cent. but the CO_2 does not rise above 9·1 per cent. There is no evidence of rapid biochemical change; it appears more probable that the CO_2 is being dissolved in the soil water.

There are still other instances where the fall in oxygen precedes the rise in CO_2: these are readily seen by inspecting the curves.

A third case presents more difficulty and has not yet been satisfactorily explained. Reference to the figures shows that several periods occur when the oxygen and CO_2 rise simultaneously: such are May–June 1913 and April 1914 on Broadbalk unmanured plot (Fig. 1), February, April and November 1913 on Broadbalk dunged plot (Fig. 2), March, April and October 1913 on Broadbalk wilderness (Fig. 3), etc. The phenomena suggest an evolution of CO_2 from the water or colloids in the soil.

In general the oxygen falls below that present in atmospheric air (20·97 per cent.) but in a few cases it exceeds this amount[1]. The occurrence is so rare that we have been unable to make a satisfactory investigation, but we incline to the view that the additional oxygen comes dissolved in the rain (p. 23). The following are instances:

		% CO_2	% O_2	% N_2
Hoos field wheat	10 Nov. 1913	0·69 0·10 0·19	21·01 21·10 21·71	78·30 78·78 78·10
Broadbalk wheat (dunged plot)	10 Nov. 1913	0·11	21·19	78·70
Geescroft	10 Nov. 1913	0·19	21·21	78·60

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[1] See also Appendix, Table XI, Hoos field fallow.

THE CAUSES OF FLUCTUATIONS OF COMPOSITION OF SOIL AIR.

A. *The variations due to season.*

These fluctuations consist in a rise to a maximum CO_2 content in late spring, a fall to a minimum in summer, a rise to a second maximum in late autumn and a fall to a minimum in winter. The oxygen content varies in the inverse sense, reaching minimum values in spring and autumn and maximum values in summer and winter.

All the curves show the same general shape when plotted over the year; proving that the effect of season completely overrides the effect of various soil treatments. Field experiments alone do not enable us to disentangle all the factors, but we took measurements for the purpose of discussing the effect of temperature and moisture content.

Effect of temperature. This can be studied from Fig 7 where the mean soil temperatures taken from the continuous recording soil thermometer are plotted along with the CO_2 in the soil air from the Broadbalk unmanured plot.

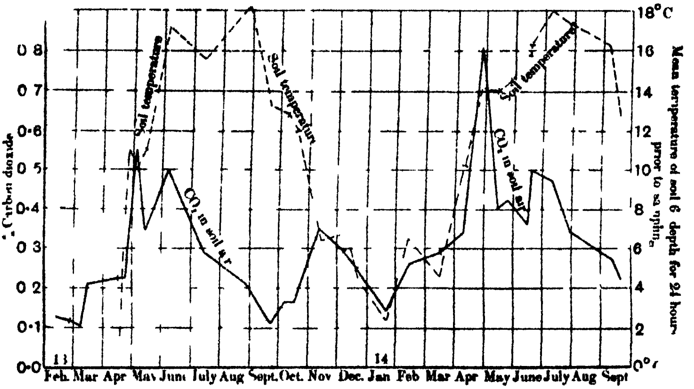

Fig 7 Curves showing percentage of CO_2 in air of Broadbalk unmanured plot and mean soil temperature (at 6″ depth) for 24 hours previous to sampling.

Beginning with the middle of April, 1913, when soil temperatures were first taken, it is seen that the temperature curve runs closely with the CO_2 curve up to the early part of May, they then part company and show no more resemblance till November. From that time, however, up to early May, there is a close general resemblance but this ceases from then onwards. Thus we can infer that the temperature is the dominating factor in determining the amounts of CO_2 production from November to May.

It is clearly not the only factor for the parallelism is not complete: a rise in temperature in spring is more potent to increase the output of CO_2 than a similar rise later on. Thus the values for temperature and CO_2 in May and June no longer show the agreement obtained earlier: the CO_2 maximum in May being above that in June while the temperature maxima fall the other way. These differences in detail indicate that other factors are operating, but they do not weaken the main conclusion that *from November to May the temperature determines the rate of* CO_2 *production in the soil*[1].

The dunged plots and the wilderness show the same general relationships, but again there are differences in detail, the CO_2 and temperature curves parting company earlier in the summer than on the unmanured plot. The main obvious difference between the plots is that the crop is larger on the dunged plot and the wilderness than on the unmanured plot, and the bearing of this factor will become evident later on.

From June to November, however, the temperature is not the main factor for the curves show no kind of similarity.

Effect of Moisture. A comparison of moisture content and CO_2 content is made in Fig. 8. The moisture determinations only began in June 1913, so that the curve does not run as long as that for temperature but it shows no connection with the CO_2 curves except during a few months in summer. The moisture is low during June, July and August of 1913 when the CO_2 is falling: it rises in September and October when the CO_2 first falls and then rises, it is steadily high from November to March 1914 during which the CO_2 first falls and then rises; it falls in April while the CO_2 rises and falls low during summer when the CO_2 also is low.

Thus moisture does not have nearly so marked an effect as temperature, and it only shows any relationship to the CO_2 during the summer months July to September.

The extreme case of water logged soil is dealt with on p. 32.

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[1] The failure to find on some of the plots a maximum CO_2 content in May 1914 of the same order as the value obtained in 1913 may be attributed to the fact that quite unwittingly we allowed a favourable temperature period to pass without taking any samples. We made determinations on May 15 and again on May 25, but during the interval there came a rise in temperature which we missed.

May 1914	15th	16th	17th	18th	19th	20th	21st	22nd	23rd	24th
Soil temperature at depths of 6 in. °C.	15·5	15·1	16·1	17·3	16·9	17·9	19·0	20·0	16·1	14·6

Rainfall. If instead of taking the percentage of moisture, we plot rainfall for the week preceding the date of sampling, we obtain a somewhat closer relationship with the CO_2 curves (Fig. 9). The May maximum (1913) is seen to coincide with a period of high rainfall: the October maximum follows after a second high rainfall and the intervening summer minimum is in a dry period: the April (1914) and the June maxima occur with other high rainfall periods. These are not simple moisture effects, for they are not brought out so clearly on the moisture curve, and we have to seek some other explanation. Two factors appear

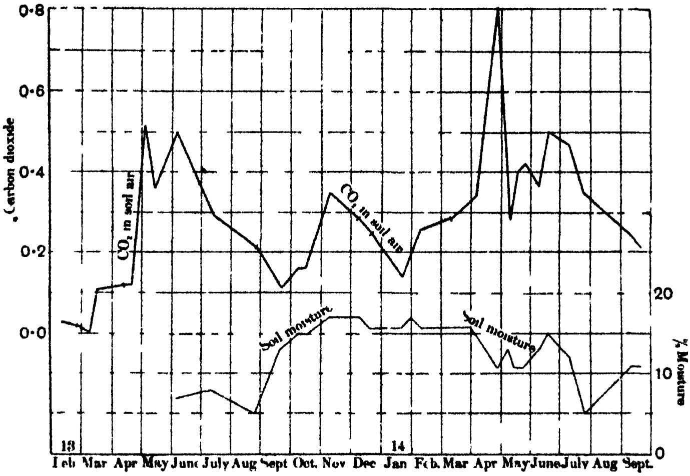

Fig 8 Curves showing percentage of CO_2 in air of Broadbalk unmanured plot and soil moisture to a depth of 9″.

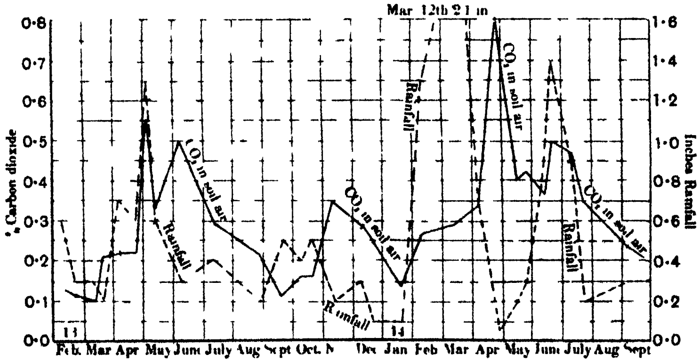

Fig 9. Curves showing percentage of CO_2 in air of Broadbalk unmanured plot, and rainfall for seven days preceding day of sampling

to come into play. In the first place the rain does not immediately distribute itself throughout the soil but produces a more or less saturated layer which seals the surface and prevents the escape of CO_2 from the soil air. Further, rain appears to be nearly saturated with dissolved oxygen. We have already seen that the dissolved atmosphere in the soil tends to lose oxygen more rapidly than to gain it and in consequence is largely anaerobic. A large fall of rain bringing with it oxygen in solution affords the possibility of partially renewing the dissolved atmosphere and giving the organisms a new lease of activity. In time, however, the oxygen is used up and the activity falls off even though the moisture remains constant. This effect is probably most marked when the soil is dry and the new dissolved atmosphere can most completely replace the old one. We could find no determinations of the amount of dissolved oxygen in rain water but a number of analyses of stream waters have been made by the Sewage Commission, and they show that on an average about ten parts per million by weight of dissolved oxygen is present. If we suppose that rain contains approximately the same amount then 1 inch of rain brings down 2¼ lbs. of oxygen per acre; this if converted into CO_2 would add 0·8 to the normal 0·2 per cent. by volume and make the total up to 1 per cent. In addition the rain itself brings down a certain amount of CO_2, but not much, and considerably less than the amount of oxygen.

Relation between soil air and atmospheric air. The experiments described in this section show that CO_2 is produced at maximum rates in spring and in autumn and at minimum rates in summer and winter. As it is constantly escaping from the soil into the atmosphere we should naturally expect to find that the CO_2 in the atmospheric air also reaches maximum amounts in spring and autumn, minimum amounts in summer and winter.

Systematic determinations of the amount of CO_2 in atmospheric air are not numerous, but those made prior to 1899 were collected by Letts and Blake in their paper already quoted[1]. A statistical examination of the data shows that, as far as they can be relied upon, they indicate an increase in atmospheric CO_2 during the period March–May, a falling off during the period May to August, and a rise during the period October to January. Thus a very close agreement is obtained with our soil results.

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[1] *Proc Roy Soc. Dublin*, 1900, 9, 107–270 and especially pp. 205 *et seq.*

B. *The effect of organic matter*

Fig. 10 shows the comparison between two plots in Broadbalk wheat field one of which is unmanured while the other receives every September a dressing of 14 tons of farmyard manure. The comparison is only strict during the winter period September to March or April when the crop is so small that it can safely be neglected; from May on to harvest time complication arises from the fact that the dunged plot carries a dense crop while the unmanured plot does not. During winter the air from the dunged plot consistently contains the larger quantity of CO_2; we can carry the strict comparison from March onwards by taking the fallow part of the dunged plot and the unmanured fallow on Hoos field, which closely resembles the unmanured plot in Broadbalk:

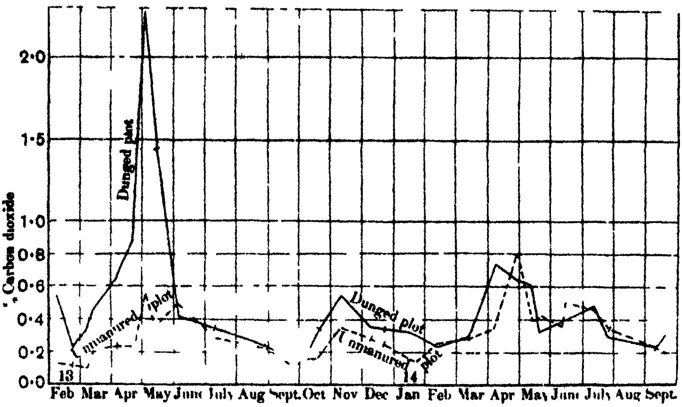

Fig 10 Comparison of CO_2 content of unmanured plot with plot receiving farmyard manure, Broadbalk field.

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Dunged fallow (Broadbalk) ..	0·22	0·32	0·17	0·36	0·36	0·36	0·35
Unmanured fallow (Hoos)	0·10	0·07	0·08	0·07	0·10	0·08	0·09

The dunged plot still gives the higher result so that the effect of the manure is clearly to increase the amount of CO_2 in the soil air throughout the year.

The persistence of this increase is its chief characteristic, and during most of the year it does not assume very great dimensions nor does it alter the shape of the curve relative to the unmanured land. The actual percentages of CO_2 during the month before and the month after ploughing in are as follows:

	Dunged plot before ploughing in	Unmanured plot		Dunged plot after ploughing in	Unmanured plot
September 22	0·17	0·11	November 10	0·54	0·35
October 6	0·18	0·16	December 9	0·35	0·29
„ 17	0·34	0·16	„ 12	0·34	0·25

Considerably larger differences however were observed during the spring both in CO_2 and oxygen in 1913 and in oxygen in 1914.

C. *The effect of a growing crop.*

As already pointed out (p. 9) there has been considerable disagreement as to the relative amounts of CO_2 in the air of cropped and of uncropped soils. Critical examination of the older work shows that much of the discussion was irrelevant because the conditions in the various experiments were not comparable. A cropped plot differs in physical state, moisture content, temperature, etc. from uncropped land and when the case is pushed to an extreme and a comparison is instituted between grass land and arable land there arises a further complication due to the difference in organic matter content of the two soils.

The usual method has been to set up a comparison between cropped and fallow portions of the same plot. We have done this in two fields. Figs. 4 and 5 and Table VI give the detailed results and Fig. 11 a simpler comparison for the Hoos wheat and fallow plots. These are made to alternate each year: the land has been unmanured since 1851 and now yields a small crop averaging 16 bushels of wheat per acre. All through the period of active growth (June to August) the cropped plot is the richer in CO_2 and it maintains its superiority even after the crop is cut and right up to the time when the land is ploughed. Then the CO_2 sinks to a low level and remains low throughout the period of fallow; it rises again as soon as the land comes into crop. The physical differences in the plots, however, are considerable. The fallow land is left rough and is not harrowed, it is occasionally cultivated to kill weeds, thus it readily allows of the escape of CO_2. The cropped land has to be got into a tilth for the seeding and it speedily becomes compact and less favourable to gaseous diffusion.

During the current year the top half of Broadbalk field has been fallowed and a comparison was made between the fallow and the

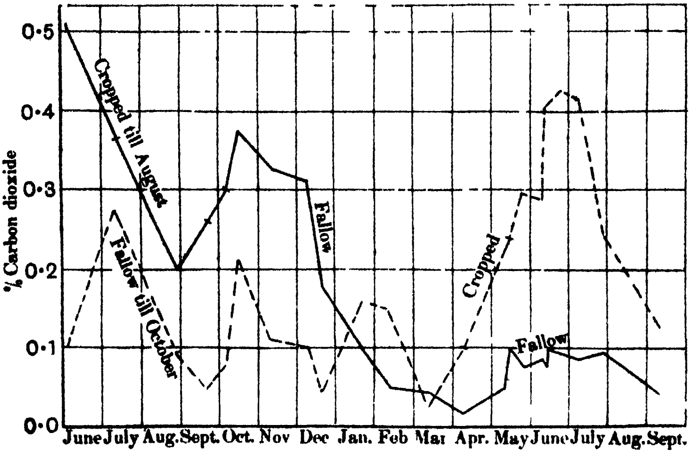

Fig. 11. Curves showing percentage of CO_2 in air of Hoos wheat and fallow plots.

cropped portions of the dunged plot. Here the conditions are different from those in Hoos field; the soil contains considerably more organic matter and does not become very compact: the difference in physical condition between the cropped and fallow portions therefore is not nearly so marked (although it still exists) and a stricter comparison is possible. Moreover the crop (which was fairly dense) did not apparently affect the temperature of the soil, and from May to July practically no differences were observed[1]. The moisture content, however, was affected, the percentage of water being:

	June 12	July 7	July 27	
Fallow portion	18	20	12	
Cropped portion	19	17	9	per cent. of water

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[1] The actual readings (6″ depth) were:

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Fallow portion ..	12°	11°	12°	12°	14°	15°	15°
Cropped portion ..	12°	11°	12°	12°	14°	15°	14°

Thus the soil conditions are still not entirely comparable but on the whole they are more so than on Hoos field. The percentages of CO_2 in the soil air were:

	May 15	May 25	June 10	June 12	June 13	July 7	July 27
Fallow portion	0·22	0·32	0·17	0·36	0·36	0·36	0·35
Cropped portion	0·61	0·32	0·35	0·48	0·42	0·48	0·30

Now the crop was considerable (30·4 bushels per acre), yet the increase in CO_2 over that in the fallow plot is not only no greater than in Hoos field but it is not usually (except on May 15) much larger than the error of experiment. Hence it appears that the effect of the growing crop in increasing the amount of CO_2 in the soil air is not great.

We can make the comparison in a different way so as to reduce in another direction the differences in physical state between the plots. The Broadbalk dunged and unmanured cropped plots both receive similar cultivations and treatment apart from manuring: both are equally exposed to the consolidating effect of the weather though the unmanured land does actually become the more closely packed. The dunged land possesses a large quantity of organic matter and carries a dense crop, both conditions favourable for a high percentage of CO_2 in soil air, yet as a matter of fact this high percentage is not obtained, and in summer when one would expect the maximum differences from the unmanured plot there is practically no difference at all[1].

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[1] On the following occasions the unmanured plot gave a higher CO_2 content than the dunged plot in Broadbalk field.

	Date	Mean composition of soil air		Moisture	Temperature °C	
		% CO_2	% O_2	Per cent in soil	Air	Soil
Unmanured plot	3 June 1913	0·50	20·77	7	22	18
Dunged plot		0·42	20·56	11		15
Unmanured plot	29 April 1914	0·81	19·98	11	10	—
Dunged plot		0·65	20·08	16		—
Unmanured plot	25 May 1914	0·42	—	11	10	12
Dunged plot		0·32	—	13		11
Unmanured plot	13 June 1914	0·50	19·80	15	21	15
Dunged plot		0·42	20·38	19		14
Unmanured plot	27 July 1914	0·35	—	5	14	16
Dunged plot		0·30	—	9		14

Determinations of the amount of CO_2 in the soil air of grass land are given in Table VII. The results show that more CO_2 is usually present than in arable land and the oxygen content is lower. But no strict comparison with arable land can be made because of the great

TABLE VII. *Composition of soil air of grassland. Percentage by volume.*

A. *Pasture used for grazing.*

Date	CO_2	O_2	N_2
Nov. 6, 1912	1·01	18·72	80·27
„ 14 „	1·59	18·12	80·29
„ 20 „	1·99	—	—
„ 21 „	1·35	—	—
„ 22 „	1·90	—	—
Dec. 2, 1913	3·34	15·18	71·48
Jan. 30, 1914	1·46	18·44	80·10
Jan. 30, 1914, 18 in. deep	1·64	17·87	80·49

B. *Geescroft Wilderness.*

Date	CO_2	O_2	N_2	CO_2	O_2	N_2	Bacterial numbers, millions per gram	N as nitrate, parts per million
Dec. 19, 1912	1·5	15·8	82·7					
Jan. 13, 1913	0·7	16·6	82·7					
Jan. 24 „	3·1	6·2	90·7					
Feb. 11 „	0·7	19·0	80·3					
Feb. 26 „	2·0	16·4	81·6					
	under *Festuca ovina*			under *Aira caespitosa*				
Mar. 13, 1913	3·9	13·0	83·1	1·1	19·4	79·5		
April 14 „	4·5	9·2	86·3	—	—	—		
April 24 „	9·1	2·6	88·3	1·3	19·1	79·6		
May 2 „	5·4	9·0	85·6	3·9	10·6	85·5		
May 13 „	7·6	8·6	83·8	3·0	14·5	82·5		
June 3 „	1·6	19·7	78·7	1·0	20·0	79·0	8	3
July 11 „	0·7	20·5	78·8	0·6	20·5	78·0	8	4
Aug. 29 „	0·4	20·5	79·1	0·4	20·5	79·1	9	4
Sept. 22 „	0·7	20·3	79·0	0·8	20·2	79·0	17	2
Oct. 6 „	0·8	20·3	78·9	0·9	20·1	79·0	—	5
Oct. 17 „	0·5	20·6	78·9	1·2	19·4	79·4	14	3
Nov. 10 „	1·0	19·7	79·3	0·9	19·8	79·3	10	1
Dec. 9 „	2·5	16·2	81·3	2·5	16·4	79·1	10	8
Dec. 22 „	1·2	19·0	79·7	2·2	16·7	81·1	17	6
Jan. 8, 1914	—	—	—	—	—	—	13	—
Jan. 20 „	1·0	19·0	80·0	0·9	19·5	79·6	8	11
Jan. 30 „	—	—	—	—	—	—	13	7
Feb. 12 „	1·8	14·2	84·0	1·6	14·8	83·6	—	6

difference in amount and composition of the organic matter present in the soil. The closest comparison we can set up is between two of the Broadbalk plots: an arable plot receiving 14 tons of dung annually and carrying each year a good crop of wheat, and an adjacent plot known as the wilderness which has remained undisturbed since 1882 and now carries a dense growth of grasses, clovers, weeds, etc., only young trees and bushes being removed. The percentages of CO_2 in the soil air are plotted in Fig. 12. There is no great difference between the two curves. In April and early May the dunged plot contains more CO_2, from September to early January it contains less, but during these months it has been ploughed up and left loosely exposed to the atmosphere for a time prior to seeding. But the differences rarely

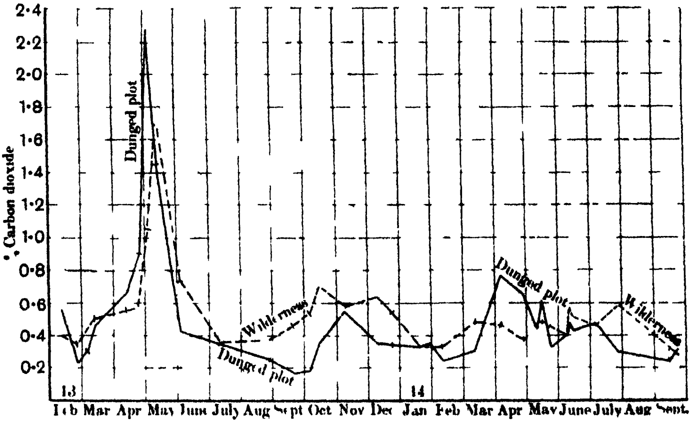

Fig. 12 Curves showing CO_2 in soil air of Broadbalk dunged and wilderness plots.

exceed 0·3 per cent. When therefore the soil conditions are comparable both as to the state of packing and to the amount of organic matter the difference between grass and arable land is less than might be expected. The result is all the more significant when it is remembered that the air of the unmanured plot is as rich in CO_2 during summer as the air of the dunged plot.

Taking them as a whole, these observations indicate that a growing crop *per se* has no very marked effect in increasing the amount of CO_2 in the soil air. Comparison is rendered difficult by the numerous differences between cropped and fallow land or between grass and arable land, which can only partially be eliminated; if an ordinary grass field is compared with an ordinary arable field considerable differences are found, but when the conditions are made more nearly alike the effect of the crop is not very great. Absolute identity of conditions has not been attained, and we cannot yet be certain whether the small effect of the crop still observed is due to uneliminated soil differences such as the removal of water by the growing crop which thus facilitates the escape of CO_2 evolved from the plant roots; or to some direct interference of the growing crop with bacterial activity in the soil.

A wholly different argument in a previous paper[1], led to the conclusion that the growing plant interferes with bacterial activity.

Before leaving this subject attention must be directed to one interesting point in connection with the two Broadbalk plots, the dunged arable and the wilderness. The arable plot shows a persistent loss of nitrogen amounting to over 100 lbs. per acre per annum, apparently not wholly by drainage. The wilderness, on the other hand, shows a persistent gain of nitrogen amounting approximately to 100 lbs. per acre per annum. We have hitherto been inclined to attribute this remarkable difference to a supposed greater aeration influencing the biochemical changes in the arable land. It is therefore of special interest to compare the oxygen content of the air from the two plots: this has been done in Fig. 13, from which it appears that there is little if any difference between them.

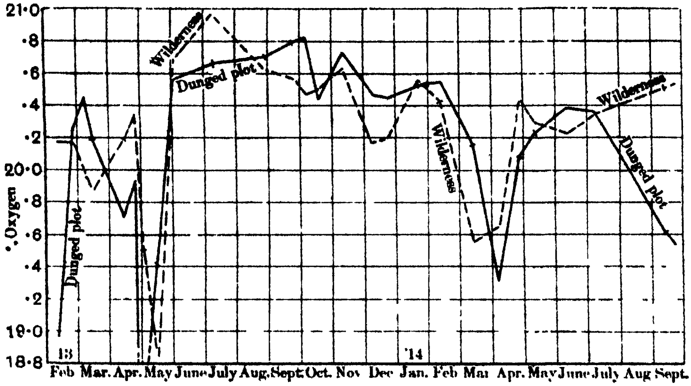

Fig. 13. Curves showing O_2 in soil air of Broadbalk dunged and wilderness plots.

Amount of CO_2 *under plants of various species.* On some of the Rothamsted grass plots and especially those that have become acid there is a tendency for certain species to segregate; determinations were therefore made of the percentage of CO_2 in the soil air of these various patches. It is found that there is a perceptible falling off of oxygen and rise in CO_2 in passing from a neutral matrix to a "sour" patch (indicated by the presence of rumex or in extreme cases by the total absence of all vegetation). But a patch of a solitary species occurring on a neutral plot such as plot 7 shows no such difference. The results are:

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[1] 'The nature and amount of the fluctuations in nitrate contents of arable soils,' *J. Agric. Sci.* 1914, 6, 18–57.

Per cent	Plot 7		Plot 5 N.		Plot 5 S.			Plot 4^2		Plot 11-1	
	Under matrix	Under spirea	Under matrix	Under dactylis	Under matrix	Under dactylis	Under dactylis and rumex	Under matrix	Under bare patch	Under matrix	Under bare patch
CO_2	1·5	1·4	1·2	1·5	1·3	1·1	2·0	2·1	1·5	1·2	2·3
Oxygen	19·3	20·0	20·0	19·7	20·0	20·1	19·5	19·0	19·5	20·1	18·5

Samples taken May 24th, 1913.

Another field where segregation occurs is Geescroft which is liable to become waterlogged in winter owing to the absence of calcium carbonate from the soil and the consequent deflocculation of the clay. During normal moist or dry conditions the soil air from the various patches is similar in composition and resembles that from the other fields. But in very wet conditions marked differences set in, the oxygen falling and the nitrogen[1] rising very considerably in amount; this happens particularly under the patches of *Festuca ovina* the roots of which form a densely matted tangle near the surface, but it is less marked under the patches of *Aira caespitosa* the roots of which form a bristly mass more readily allowing gaseous diffusion. The results are plotted in Fig. 6, they are as follows:

Wet conditions

1913	% CO_2		% O_2		% N_2	
	Aira	Festuca	Aira	Festuca	Aira	Festuca
March 13	1·1	3·9	19·4	13·0	79·5	83·1
April 14	—	4·5	—	9·2	—	86·3
April 24	1·3	9·1	19·1	2·6	79·6	88·3
May 2	3·9	5·4	10·6	9·0	85·5	85·6
May 13	3·0	7·6	14·5	8·6	82·5	83·8

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[1] Examination for hydrogen or methane has so far led to negative results.

Dry conditions

1913	% CO_2		% O_2		% N_2	
	Aira	Festuca	Aira	Festuca	Aira	Festuca
June 3	1·0	1·6	20·0	19·7	79·0	78·7
July 11	0·6	0·7	20·5	20·5	78·9	78·8
August 29	0·4	0·4	20·5	20·5	79·1	79·1
September 22	0·8	0·7	20·2	20·3	79·0	78·9
October 6	0·9	0·8	20·1	20·3	79·0	78·9

The low amount of CO_2 relative to the oxygen used up has already been discussed (p. 19).

Minor fluctuations in composition of the soil air.

We now turn to a consideration of the minor fluctuations in composition of the soil air. These differ fundamentally from the major fluctuation hitherto dealt with in as much as they are probably not associated with the production of CO_2 in the soil but only with variations in the agencies causing loss. They are brought about by two causes:

(1) Variations in the soil itself: shown in Table XI (p. 41) and discussed on p. 4.

(2) Variations in meteorological and cultivation conditions.

The only satisfactory way of dealing with the effect of meteorological conditions on the soil atmosphere is by statistical methods. but although we have many records we do not feel that they are sufficiently numerous for the purpose. We have, however, tested certain broad and obvious possibilities, the data for which are found in Table VI (p. 46).

(*a*) *Rapid change of temperature.* It has happened on a warm day preceded by a frosty night, *i.e.* where the temperature altered quickly and considerably, that the soil air approximated closely in composition to atmospheric air indicating that it had been largely replaced by atmospheric air. Instances occur on January 13th and February 26th, 1913.

(*b*) *High rainfall.* In view of the quantity of bicarbonates in drainage water it is important to ascertain whether high rainfall appreciably diminishes the amount of CO_2 in the soil air. The observations do not yield any very definite results: in some cases the immediate effect is to reduce the CO_2 but not always, while usually the subsequent effect is to increase it (p. 23, Fig. 9). The following data serve as illustrations:

Date	June 10th	June 12th	June 13th
Rainfall of previous 24 hours	0·33 in.	—	0·65 in.
CO_2 per cent. in soil air:—			
Broadbalk unmanured plot	0·36	0·37	0·50
„ dunged plot	0·40	0·48	0·43
„ wilderness	0·40	0·58	0·51
Hoosfield wheat	0·28	0·41	0·43
„ fallow	0·08	0·07	0·10

These observations confirm the older results of Fodor[1].

(1) *Strong winds.* On several occasions, *e.g.* February 3rd, March 7th, 1913, samples were taken directly after a windy night but there was nothing at all to indicate that the composition of the air had been affected by the wind. A current of air passing rapidly over the soil might have been expected to draw out the soil air, but apparently it does not. Probably the force is insufficient, the layer of air in contact with the surface of the soil moves less quickly than the layers a few inches above. Moreover any removal of air by this process from the surface layers of the soil probably leads to an upward movement of air rich in CO_2 from the lower depths.

(2) *Change in barometric pressure.* Fodor[2] found that the CO_2 in soil air rose with falling barometer at three stations out of four where investigations were made. In the only continuous experiment we made we were fortunate in happening upon a time when the barometer was rapidly falling and we also obtained a rise in CO_2 during the period. But when the whole of our CO_2 figures are plotted against barometic pressures or even against changes in barometric pressure no consistent relationship can be observed such as is obtained with rainfall, temperature, etc., so that the influence of barometric pressure appears to be only minor and easily swamped by other factors.

(3) *Night and day.* Fodor[3] and Wollny[4] thought they had evidence that CO_2 streams out from the soil air at night but we can find no indication of any greater loss by night than by day. Samples drawn from the same 5 holes at consecutive 3-hour intervals over a period of

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[1] Josef Fodor, *Hygienische Untersuchungen über Luft, Boden und Wasser*, Braunschweig, 1881, p. 130.

[2] Fodor, *ibid.* p. 135.

[3] Fodor, *ibid.* p. 53.

[4] Wollny, *Forsch. auf dem Gebiete der Agrik.-Physik*, 1885, S. 417.

33 hours failed to show any systematic variation as between the day and the night. The results are given in Table VIII. The CO_2 tends to rise and the oxygen to fall from the 18th hour onwards (*i.e.* from 3.30 a.m. on the 15th) when the barometer is steadily falling, but there is no sign of any relationship with the temperature either of the air or the soil.

TABLE VIII. *Hourly fluctuations in composition of soil air, 3-hour periods over 33 consecutive hours.*

Hour	0	3	6	9	12
	A.M.	P.M.	P.M.	P.M.	P.M.
Time Nov. 14 ..	9.30	12.30	3.30	6.30	9.30
% CO_2 (mean) ..	0·11	0·13	0·11	0·15	0·13
% O_2 (mean) ..	20·69	20·82	20·65	20·70	20·61
Barometer mm. ..	742	746	747	748	749
Air temp. °C. .	5	6	2	−1	−2
Soil temp. °C. ..	2	6	6	5	5

Hour	15	18	21	24	27	30	33
	A.M.	A.M.	A.M.	A.M.	P.M.	P.M.	P.M.
Time Nov 15	12.30	3.30	6.30	9.30	12.30	3.30	6.30
% CO_2 (mean)	0·13	0·13	0·14	0·16	0·16	0·19	0·18
% O_2 (mean) ..	20·61	20·62	20·52	20·54	20·51	20·42	20·43
Barometer mm.	747	744	738	733	731	730	729
Air temp. °C. ..	0	1	1	1	13	8	6
Soil temp. °C. ..	5	5	5	5	7	6	5

(4) *Cultivation.* We have not made systematic investigations into the effects of the various cultivation operations, but we find that ploughing usually increases the percentage of oxygen and diminishes the CO_2 in the soil air, the fall in CO_2 being particularly marked when the ploughing is done early. The details are given in Table IX, where also are set out the analytical data for the uncultivated wilderness.

The relation between carbon dioxide production, nitrate formation and bacterial numbers.

The curves showing the amounts of carbon dioxide in the soil air and of nitrate in the soil are so similar in character as to justify the view that both essentially represent the rates of formation (p. 12). Closer comparison of the curves with those for bacterial numbers brings out several important features which we must now proceed to discuss.

Fig. 14 shows the rainfall, bacterial numbers, carbon dioxide and nitrate for the Broadbalk dunged plot, which is perhaps the most convenient for our purpose by reason of the high values it yields. Beginning in July, 1913, the bacterial numbers follow the rainfall very closely till October and less closely till January, the diminishing rainfall of July and August being accompanied by a fall in bacterial numbers, the September rain by a rapid rise, and so on. The CO_2 curves also follow in the same way but later in point of time and they are somewhat smoothed out: thus they do not show the kink in October. The nitrate curves again show the same rise but still later; in comparing them with the others, however, it must be remembered that conditions of drought which favour a decrease of bacteria through death and of CO_2 through diffusion have no effect in reducing the amounts of nitrate: thus during

TABLE IX. *Percentage composition of soil air before and after cultivation operations.*

Date	Uncultivated land		Cultivated land					
	Wilderness		Broadbalk Dunged		Broadbalk Unmanured		Hoos Fallow Wheat	
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2
July 11 ..	0·36	20·97	0·35	20·66	0·29	20·79	0·27	20·66
			Wheat cut		Wheat cut		Ploughed	
August 29 ..	0·37	20·62	0·24	20·70	0·22	20·73	0·09	20·84
			Ploughed and harrowed		Ploughed and harrowed			
September 22	0·46	20·57	0·17	20·79	0·11	20·83		—
October 6 ..	0·53	20·47	0·18	20·81	0·16	20·82		—
					Ploughed			
October 17 ..	0·70	20·50	0·34	20·43	0·16	20·72	0·21	21·30
			Ploughed, harrowed and drilled		Harrowed and drilled		Drilled with wheat	
November 10	0·58	20·62	0·54	20·72	0·35	20·56	0·11	20·90
December 22	-	—	—	—	—	—	0·17	20·44
							Ploughed	
January 20 ..	—	—	—	—	—	—	0·10	20·92

July and August the nitrates increase instead of falling like the CO_2. But in November and December the nitrates rise sharply and keep high until the heavy February rains[1], when they fell to a minimum just as do the bacterial numbers and the carbon dioxide.

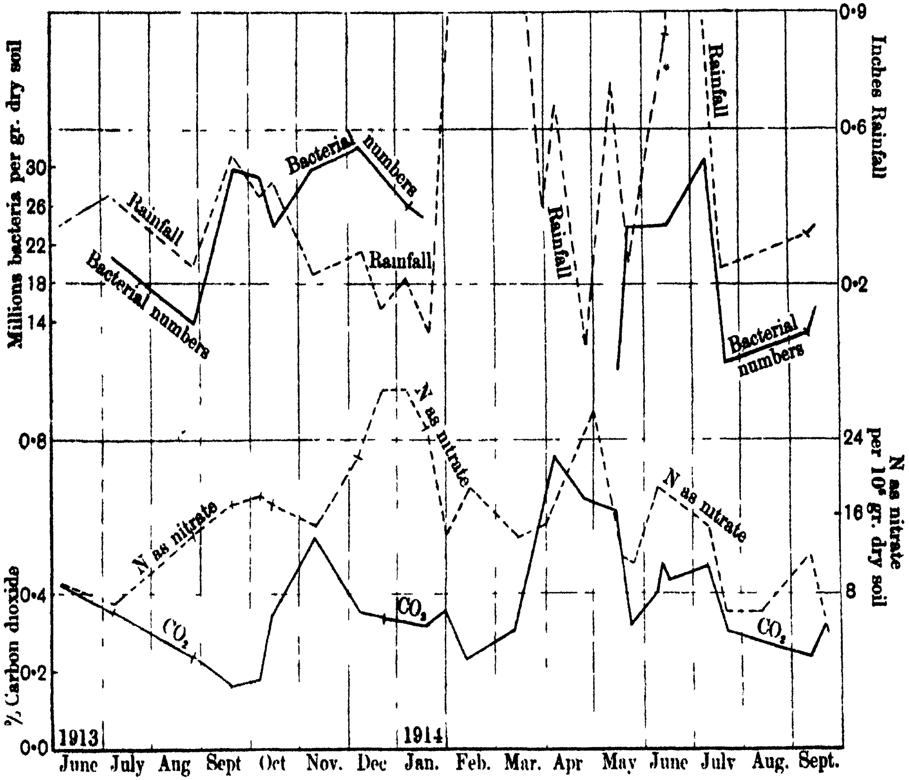

Fig. 14. Curves showing rainfall, bacterial numbers in soil, CO_2 in soil air and nitrate in soil of Broadbalk dunged plot.

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[1] The rainfall for December 1913 and January 1914 was considerably below the average so that the washing out of nitrate began later than usual. The rainfall figures are:

	December	January
Average 1853–1913	2·44	2·35
This year ..	0·88	0·88

It is interesting to note that, when the drains began to run in February 1914, the drainage water was of approximately the same order of concentration as after the similar winter conditions of 1879–80:

N as nitrate in drainage water from Plot 2 (dunged)

February 1914	26·8	29·7	parts per million
„ 1880	27·3		„ „

Unfortunately there was a break in the bacterial counts during the winter months, but the other observations were made. In March, 1914, there occurred a high rainfall, followed by a rise in CO_2 and somewhat later by a rise in nitrate: in April the CO_2 falls, but in May and June there is a sharp increase in rainfall and in bacterial numbers, followed by an increase of CO_2 and of nitrate.

If we take the unmanured (Fig. 1) instead of the dunged plot we obtain similar but numerically smaller results. The wilderness (Fig. 3) also shows the same general phenomena, but the spring rise in the nitrate is considerably flattened down in consequence of the rapid absorption by the plants; the autumn rise, however, is seen, and as before it comes after the rise in CO_2 and this in turn after the rise in bacteria. Again, the Hoos wheat and fallow plots (Figs. 4 and 5) show like similarity between bacterial numbers, CO_2 and nitrates, especially during the fallow period The fluctuations are not great—the land having received no manure for many years is very impoverished—and it would be unsafe to attach too much importance to some of them, but they all go in the same direction. During the time when the land carries a crop of wheat (Fig 5) the nitrate curve is flattened from April to July; while on the other hand the loosening of the land during the fallow period causes a flattening of the CO_2 curve

The general conclusion is that the fluctuations in bacterial numbers, in CO_2 content and in nitrates in the soil are all of the same general character, and this character is mainly impressed by seasonal factors other conditions such as manuring, cropping, etc , may pull out or flatten the curves but they do not alter their general shape The production both of nitrates and of CO_2 attains a maximum in late spring or early summer, a minimum in summer, a maximum in late autumn and a minimum in winter[1], the numbers of bacteria fluctuate in the same way in summer, autumn and winter. When the autumn rains came after the dry summer conditions, the bacteria immediately responded by rapid multiplication: then there came an increase in the amount of CO_2 in the soil air and finally an increase in the amount of nitrates. This order seems to be pretty general.

The spring and autumn periods of maximum biochemical activity in the soil are clearly of great significance in soil management

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[1] Similar seasonal fluctuations in nitrate content are recorded in the paper already quoted in *J. Agric. Sci.* 1914, **6**, 18–57.

APPENDIX.

I. *Method of collecting and analysing the soil air.*

The apparatus used for collecting the soil air is shown in Fig. 15; it was used by Hall and Russell in their investigations of the air of Romney marsh soils. It consists of a hollow cylindrical steel tube (*A*) 2 feet long, ⅝ in. outside and ⅜ in. inside diameter to which is welded a side tube (*R*) 2½ inches from the top to allow of the air being withdrawn from the nozzle (*S*). The top of the tube is strengthened by a cap (*B*). A solid cylindrical rod (*N*) ⅜ in. in diameter with a flat side ⅛ in. wide running its whole length fits tightly into the hollow tube; it is provided at the bottom with a collar ⅛ in. wide.

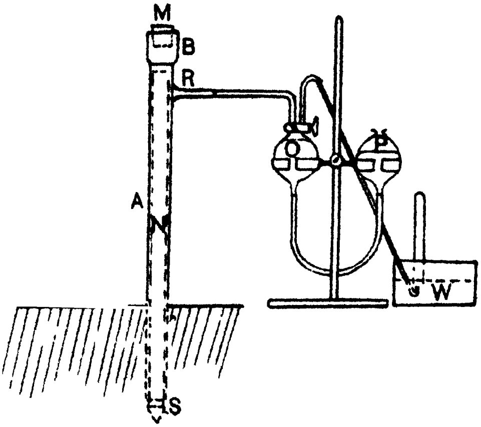

Fig. 15. Apparatus for the collection of soil air.

To obtain a sample of soil air the tube is driven vertically down into the soil to the required depth with a wooden mallet, great care being taken to prevent lateral movements. The inner rod (*N*) is then punched down about ¼ in. and a rubber stopper (*M*) inserted in the hole at the top of the tube.

A small bulb (*O*) of approximately 30 c.c. capacity provided with a two way tap is connected to the side tube (*R*) by means of pressure tubing, and also to a small mercury reservoir (*P*); it has a delivery tube attached through which the gas is forced into a mercury trough (*W*) for collection. The flat side of the inner rod allows the gas to pass freely up the tube when the pressure in the bulb is diminished by lowering the mercury reservoir. The first 20–30 c.c. is rejected and the next 25 c.c. is collected over mercury in thick-walled test tubes, which are then placed in small crucibles and transported in a rack to the laboratory for analysis. To prevent the rack from being blown over by winds it is held firmly in the ground by iron spikes passing through the base pieces. Only one sample is collected at each point. Successive samples vary slightly in composition (Table X) but a fairly large volume of air of tolerably uniform composition can if desired be withdrawn from the same hole.

TABLE X. *Percentage composition of successive* 30 *c.c. samples of soil air drawn from the same hole.*

Hole 1	CO_2	O_2	N_2	Hole 2	CO_2	O_2	N_2
1	0·10	20·74	79·16	1	0·36	20·36	79·28
2	0·10	20·72	79·18	2	0·45	20·46	79·09
3	0·11	20·86	79·03	3	0·39	20·55	79·06
4	0·12	20·63	79·25	4	0·36	20·54	79·10
5	0·14	20·77	79·09	5	0·36	20·57	79·07
6	0·12	20·67	79·21	6	0·36	20·45	79·19
7	0·12	20·71	79·17				
8	0·13	20·80	79·07	Hole 3			
9	0·13	20·68	79·19	1	0·18	20·62	79·20
10	0·13	20·79	79·08	2	0·18	20·74	79·08
11	0·13	20·69	79·18	3	0·15	20·63	79·22
12	0·13	20·76	79·11	4	0·15	20·74	79·61
				5	0·18	20·74	79·08
				6	0·23	20·54	79·23
				Hole 4			
				1	0·26	20·57	79·17
				2	0·25	20·49	79·26
				3	0·25	20·45	79·30
				4	0·23	20·52	79·25
				5	0·23	20·74	79·03
				6	0·21	20·63	79·36

As a rule samples of air from 8–12 holes on each plot are drawn and analysed separately, and the mean value is taken to represent fairly accurately the composition of the soil air. These mean values are given in Table VI and plotted in the various Figures 1 to 6.

Samples were drawn from all the plots on the same day so that the values are strictly comparable. The variation from place to place is fairly large, especially on the plot which has received annually 14 tons of farmyard manure, but on the unmanured plot it is comparatively narrow.

TABLE XI. *Showing variation in percentage composition of soil air taken from different holes on the same plot.*

Hole	Broadbalk (dung) CO_2	Broadbalk (dung) O_2	Broadbalk (dung) N_2	Hole	Broadbalk (unmanured) CO_2	Broadbalk (unmanured) O_2	Broadbalk (unmanured) N_2
1	0·39	20·63	78·98	1	0·27	20·69	79·04
2	0·32	20·66	79·02	2	0·19	20·77	79·04
3	0·25	20·76	78·99	3	0·33	20·63	79·04
4	0·37	20·69	78·94	4	0·29	20·64	79·07
5	0·34	20·70	78·96	5	0·38	20·67	78·95
6	0·32	20·69	78·99	6	0·34	20·69	78·97
7	0·41	20·53	79·06	7	0·29	21·09	78·62
8	0·40	20·54	79·06	8	0·26	21·15	78·59
Mean	0·35	20·65	79·00		0·29	20·79	78·92
Probable error of 1 determination	±0·03	±0·06			±0·03	±0·11	
Probable error of mean of all 8	±0·01	±0·02			±0·02	±0·05	

Hoos Field Fallow, Oct. 17, 1913.

Hole	CO_2	O_2	N_2
1	0·19	22·33	77·48
2	0·18	21·27	78·55
3	0·25	21·13	78·62
4	0·34	21·09	78·57
5	0·12	21·12	78·76
6	0·20	21·26	78·54
7	0·12	21·19	78·69
8	0·25	20·99	78·76
Mean	0·21	21·29	78·50
Probable error of 1 determination	±0·04	±0·16	
Probable error of mean of all 8	±0·02	±0·10	

It has frequently been found impossible to obtain a sample of air when the steel rod is driven into the clay subsoil and also when the surface of the ground is frozen. The soil was very wet and the pore space comparatively small, and displacement of the soil air apparently could not take place.

Analysis of soil air. The large type of Haldane's gas apparatus is used. The measuring tube (*A*, Fig. 16) has a capacity of 21 c.c. and is graduated from 15–21 c.c. into 0·01 c.c. The analysis of the gas is carried out under constant pressure; temperature and water vapour pressure are compensated by the bulb shown to the left. The water in the jacket must be thoroughly mixed before readings are taken: this is done by blowing though it air from foot bellows. A laboratory vessel (*B*) in a porcelain mercury trough (*C*) [as used in the well-known Bone and Wheeler gas apparatus] is attached to the measuring tube and filled with mercury by connecting with an evacuated flask (*D*) provided with a mercury trap (*S*). Through this laboratory vessel the gas is readily introduced into the measuring tube. The analysis proceeds in the usual way. Finally the residual gas is forced by means of the levelling tube (*E*) into the laboratory vessel and ejected. A small telescope

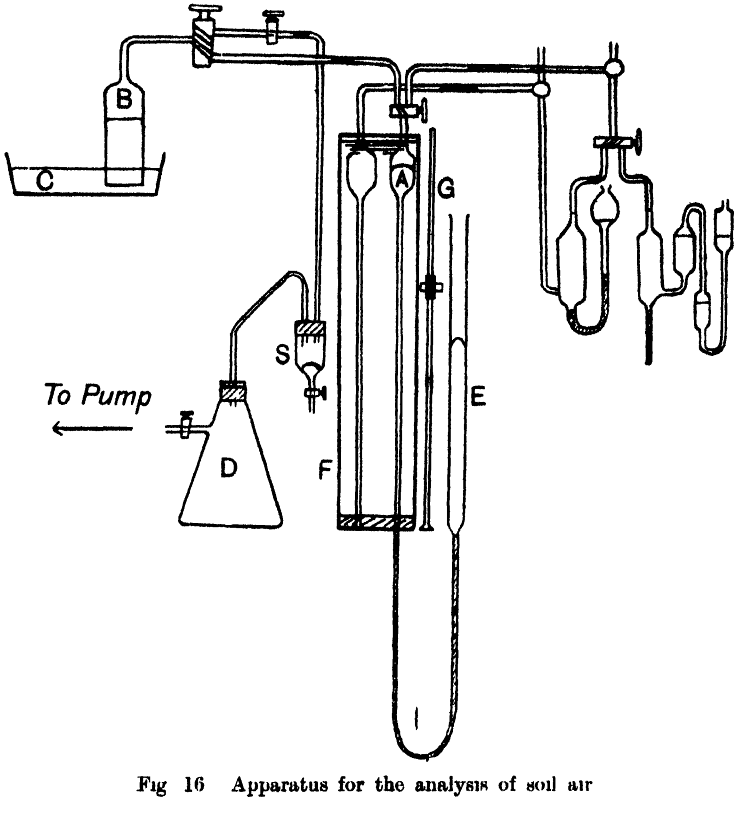

Fig 16 Apparatus for the analysis of soil air

sliding on a fixed brass rod (*G*) in front of the water jacket (*F*) which surrounds the measuring tube, and an electric light behind, enable accurate readings to be taken. The laboratory vessel also allows of analyses being made by absorption with small quantities of reagents.

A simple apparatus for teaching purposes. For teaching and demonstration purposes the apparatus in Fig. 17 is very useful. A piece of half inch gas pipe is driven 6 inches into the soil and connected through a tube of baryta water to a large bottle of water fitted with a syphon tube so that it can act as an aspirator. A second tube of baryta water open to the atmosphere is also attached to the bottle. Set the aspirator going and arrange the clips so that air bubbles pass through both baryta tubes at the same rate. In a short time the one connected with the soil shows turbidity while the other open to the air is still clear.

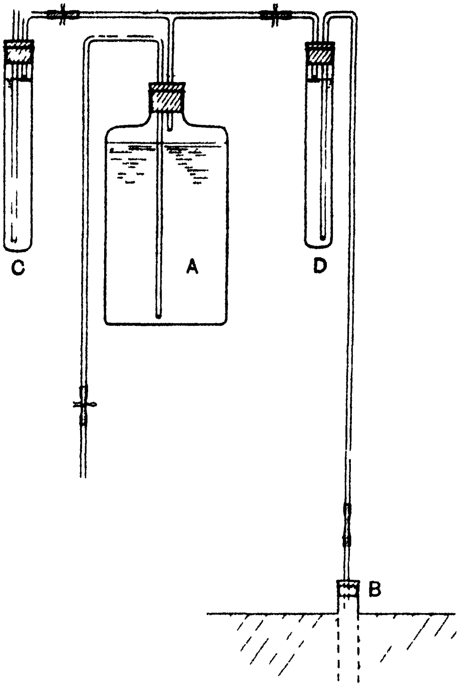

Fig 17 Apparatus for demonstrating the pressure of CO_2 in soil air

A, aspirator *B*, ½" gas pipe driven into soil.
C, tube of saturated baryta water open to air
D, " " " " connected to soil

II. *The soil of the Rothamsted fields.*

All the samples of air dealt with in this paper have been drawn from the Rothamsted fields. The soil is a heavy loam with many stones: it becomes very sticky when wet, but can be got into a good crumbly tilth as it becomes drier. Its mechanical analysis is as follows:

Top 9 inches.

Name of fraction	Diameter of particles	Broadbalk %	Hoos Field %
Fine gravel	3 to 1 mm.	1·9	2·0
Coarse sand	1 to 0·2 mm.	6·2	6·8
Fine sand	0·2 to 0·04 mm.	21·4	19·5
Coarse silt	0·04 to 0·01 mm.	32·5	28·9
Fine silt	0·01 to 0·002 mm.	13·8	15·5
Clay	less than 0·002 mm	17·6	18·8

The pore space and space normally occupied by air are

Soil from	Loss on ignition, %	Specific gravity of dry soil		Volume occupied in natural state by		Volume of water		Volume of air	
		Apparent	True	Solid matter	Air and water pore space	In normal moist state	After period of drought	In normal moist state	After period of drought
Broadbalk unmanured plot	4·3	1·57	2·36	65·9	34·1	23·2	17	10·9	17·1
Broadbalk dunged plot	10	1·46	2·31	61·8	38·2	30·3	20	7·9	18·2

SUMMARY AND CONCLUSIONS.

1. The free air in the pores of the soil to a depth of 6 inches is very similar in composition to the atmospheric air but it differs in two respects:

(*a*) It contains more CO_2 and correspondingly less oxygen, the average in 100 volumes being 0·25 volume CO_2 and 20·6 of oxygen against 0·03 volume CO_2 and 20·96 oxygen in atmospheric air.

(*b*) It shows greater fluctuations in composition.

Usually the sum of the CO_2 and oxygen is only slightly less than in atmospheric air but at periods when nitrates rapidly increase there is a perceptible falling off of oxygen, and a still greater one in waterlogged soils.

2. Besides this free air there is another atmosphere dissolved in the water and colloids of the soils. This consists mainly of CO_2 and nitrogen and has practically no oxygen.

3. The fluctuations in composition of the free soil air are mainly due to fluctuations in the rate of biochemical change in the soil, the curves being similar to those showing the amount of nitrate and the bacterial counts as far as they were taken. The rate of biochemical activity attains a maximum value in late spring and again in autumn, and minimum values in summer and winter. In autumn the bacteria increase first, then the CO_2 rises, and finally the nitrate increases.

From November to May the curves closely follow those for the soil temperature which thus appears to be the dominating factor; from May to November they follow the rainfall and to a less extent the soil moisture curves. The distinct difference between rainfall and soil moisture indicates that rainfall does something more than add water to the soil. It is shown that the dissolved oxygen brought in is probably a factor of considerable importance in renewing the dissolved soil atmosphere and facilitating biochemical change.

4. Grass land usually contains more CO_2 and less oxygen than arable land but we cannot attribute the difference to the crop owing to the large differences in soil composition and conditions. It is difficult to ascertain the precise effect of a crop, but as the soil differences are eliminated so the differences in composition of the soil air become less and less. No evidence could be obtained that the growing crop markedly increases the amount of CO_2 in the soil air, and if it gives rise to any great evolution of CO_2 in the soil it apparently exercises a corresponding depressing effect in the activities of soil bacteria. This result agrees with one obtained earlier in reference to the nitrates in the soil.

5. Such weather conditions as barometric pressure, wind velocity, variations in temperature from the mean, small rainfall, etc. seem to have but little effect on the soil atmosphere.

TABLE VI (*a*). *Composition of soil air (mean percentages by volume) and meteorological data.*

Date	Uncultivated land Broadbalk Wilderness			Arable land												Meteorological data				
				Broadbalk wheat						Hoos wheat and fallow										
				Dunged			Unmanured			(1)			(2)			Rain-fall for 7 previous days	Mean temps for previous day		Baro. mms.	Character of previous day
	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2		air	soil 6"		
1912 Dec. 19	0·55	20·35	79·10	—	—	—	—	—	—	—	—	—	—	—	—	0·38"	4·4° C.	—	748·5	Showery and dull till midday; then fine and frosty at night.
1913 Jan. 24	0·28	20·40	79·32	—	—	—	—	—	—	—	—	—	—	—	—	1·22	4·4	—	748·7	Dull; snow melted by 9.0 a.m.; much milder; S. wind.
Feb. 3	0·40	20·45	79·15	—	—	—	—	—	—	—	—	—	—	—	—	0·14	4·4	—	752·2	Fair morning, sharp rains in afternoon and early evening; S.W. winds.
Feb. 11	0·40	20·17	79·43	0·55	18·97	80·48	0·13	19·86	80·01	—		—	—	—	—	0·60	8·3	—	766·2	Fair, bright in afternoon; S.W. winds.
	0·33	20·16	79·51	0·22	20·24	79·54	0·12	20·72	79·16	—		—	—	—	—	0·30	4·8	—	748·0	Fair till midday; little rain at noon; fair; S.E. light winds.
Mar. 7	—	—	—	0·31	20·44	79·25	0·10	20·77	79·13	—		—	—	—	—	0·33	10	—	753·2	Fair day, some rain with gusty winds at night.
Mar. 13	0·50	19·87	79·63	0·46	20·18	79·36	0·21	20·53	79·26	—	—	—	—	—	—	0·22	3·9	—	754·2	Fine, light S. winds.
Apr. 14	0·55	20·19	79·26	0·65	19·70	79·65	0·22	20·64	79·14	—	—	—	—	—	—	0·68	1·7	—	754·7	Fair, cold N. winds, bright sunshine at times.
Apr. 24	0·57	20·34	79·09	0·89	19·93	79·18	0·22	20·71	79·07	—	—	—	—	—	—	0·56	11·1	—	749·2	Fine, warm, light E. winds.
May 2	1·04	19·50	79·46	2·27	17·61	80·12	0·55	20·19	79·26	—	—	—	-	—	—	1·29	10	—	750	Fair generally; showery evening; fine frosty air at night; S. to S.W. winds.
May 13	1·73	18·82	79·45	1·45	19·42	79·13	0·35	20·53	79·12	—		—	—	—	—	0·56	10·5	11·1	756·2	Fair morning, showery from noon onwards till evening.
June 3	0·72	20·69	79·59	0·42	20·56	79·02	0·50	20·77	78·73	0·51	39	79·10	0·10	20·82	79·08	0·35	13·9	17·4	755·5	Fine; S. winds.
July 11	0·36	20·97	78·67	0·35	20·66	79·01	0·29	20·79	78·92	0·30	20·64	79·00	0·27	20·66	79·07	0·42	15	15·5	752·2	Dull; mild; thunder in S. from 1.30 to 3 p.m.; some showers; fine later.
Aug. 29	0·37	20·62	79·01	0·24	20·70	79·06	0·22	20·73	79·05	0·20	20·70	79·10	0·09	20·84	79·07	0·24	19·4	18·1	750	Fine hot morning; light N.E. wind; fine and very hot.
Sept. 22	0·46	20·57	78·97	0·17	20·79	79·04	0·11	20·83	79·06	0·26	20·80	78·94	0·05	21·01	78·94	0·52	14·4	13·3	757·2	Fine and bright; warmer N.W. wind very light; little fog at night.
Oct. 6	0·53	20·47	79·00	0·18	20·81	79·01	0·16	20·82	79·02	0·30	20·47	79·23	0·07	20·85	79·08	0·41	11·1	12·9	745	Changeable, sprinkles of rain in afternoon; S. breezes.
Oct. 17	0·70	20·50	78·80	0·34	20·43	79·23	0·16	20·72	79·12	0·38	20·61	79·01	0·21	21·30	78·49	0·47	12·2	12·0	759	Fair, light W. winds; good deal of cloud in afternoon and evening; misty at night.
Nov. 10	0·58	20·62	78·80	0·54	20·72	78·74	0·35	20·56	79·09	0·33	21·06	78·61	0·11	20·90	78·99	0·21	5·0	6·5	744·7	Fine; S.W. light airs, some rain at night.

Dec. 9	0·64	20·17	79·19	0·35	20·47	79·18	0·29	20·27	79·44	0·32	20·57	79·11	0·10	20·76	79·14	0·29	7·2	6·9	755	Dull, mild, damp at times; S.E.
Dec. 22	0·53	20·19	79·28	0·34	20·45	79·21	0·25	20·35	79·40	0·17	20·44	79·39	0·04	20·68	79·28	0·13	2·8	4·5	763·5	Dull: slightly misty; E. light breezes; slight frost at night.
1914 Jan. 20	0·32	20·55	79·13	0·32	20·51	79·17	0·14	20·62	79·24	0·10	20·92	78·98	0·16	20·88	78·96	0·09	0·5	2·4	753·5	Dull, cheerless day, cold N.E. winds; snow midday; sleet in evening.
Jan. 30	—	—	—	0·34	20·52	79·14	—	—	—	—	—	—	—	—	—	0·19	7·2	4·6	751·7	Fair, mild, light S. breezes, sprinkle of rain about 1 p.m.
Feb. 12	0·32	20·42	79·26	0·24	20·54	79·22	0·26	20·33	79·41	0·04	20·85	79·11	0·15	20·77	79·08	1·27	7·8	6·5	743·5	Dull with rain all day; heavy showers at night; S. to S.E.
Mar. 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·07	6·1	5·8	753·2	Fair day, cool W. winds; shower about 7.30 p.m.; then fair again.
Mar. 11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1·72	3·3	4·7	751·5	Fine morning; cold; snow; fine later; sharp white frost.
Mar. 12	0·49	19·56	79·95	0·30	20·16	79·54	0·29	20·28	79·43	0·03	20·69	79·28	0·03	20·75	79·22	2·12	3·3	4·7	744·2	Fair or fine during day; rain at night; W. winds.
Mar. 31	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·40	8·9	7·4	739	Showery; W. winds.
April 6	0·48	19·65	79·87	0·76	19·31	79·93	0·34	19·85	79·81	0·02	20·78	79·20	0·10	20·58	79·32	0·68	8·9	10·1	741·2	Fair during the day; heavy showers from 7 p.m. onwards; W. wind.
April 29	0·38	20·44	79·18	0·65	20·08	79·27	0·81	19·98	79·21		—	—	—	—	—	0·04	10	14·1	756	Fine and bright; E. airs cool at night.
May 11	0·45	20·29	79·26	0·44	20·22	79·34	0·28	20·45	79·27	0·05	20·73	79·22	0·18	20·48	79·34	0·71	6·1	9·1	752·2	Dull and cold; drizzling rain at times; W. breezes light.
May 15	0·48	—	—	0·61	—	—	0·40	—	—	0·10	—	—	0·24	—	—	0·16	12·8	14	760·7	Fine: warmer; S.W.
May 25	0·46	—	—	0·32	—	—	0·42	—	—	0·07	—	—	0·29	—	—	0·26	10	14·6	760·2	Fair generally, cold, some showers in afternoon; N.W. to N.
June 10	0·40	—	—	0·40	—	—	0·36	—	—	0·08	—	—	0·28	—	—	0·84	11·7	15·8	749	Fair morning and mild; dull later; colder continuous rain after 7 p.m.; N.W.
June 12	0·58	—	—	0·48	—	—	0·37	—	—	0·07	—	—	0·41	—	—	1·49	13·3	16·3	752·2	Fine, fresh S. to S.E. breezes till 3 p.m.; then heavy rain till 8 p.m. ; N. to N.W.
June 13	0·51	20·21	79·28	0·43	20·39	79·18	0·50	19·80	79·70	0·10	20·62	79·28	0·43	19·89	79·68	1·38	17·2	16·1	753·2	Dull, misty at early morn; bright periods in afternoon; N.W. light breezes.
July 7	0·47	20·35	79·18	0·48	20·36	79·16	0·47	20·29	79·24	0·08	20·54	79·38	0·42	20·15	79·43	0·88	14·4	18	752·2	Showery at times, S. to S.W. light breezes; sharp shower about 7 p.m.
July 27	0·58	—	—	0·30	—	—	0·35	—	—	0·09	—	—	0·24	—	—	0·25	15·0	17·4	743·5	Cool; good deal of cloud; one or two showers between 2 and 4 p.m.
Sept. 12	0·32	20·50	79·18	0·24	20·61	79·15	0·24	20·54	79·22	0·04	20·72	79·24	0·13	20·70	79·17	0·32	15·0	16·2	750·5	Showery during morning, fine later, cool at night; S. wind.
Sept. 21	0·28	20·52	79·20	0·31	20·53	79·16	0·22	20·60	79·18	Up to Oct. 6, 1913 (1) is Hoos Wheat and (2) Hoos Fallow. After Oct. 6, 1913 (1) is Hoos Fallow and (2) Hoos Wheat. Hoos Wheat of 1913 ploughed up between Dec. 22, 1913 and Jan. 20, 1914 for the 1914 fallow.						0·33	10	12·2	760·7	Fair generally; slight showers about noon and 6 p.m.; cold N.W. winds.

TABLE VI (*b*). *Moisture content, nitrate content, and bacterial numbers in soils on dates of sampling.*

Date	Soil moisture per cent.					N as nitrate, parts per million					Bacterial numbers. Millions per gram				
	Broadbalk			Hoos		Broadbalk			Hoos		Broadbalk			Hoos	
	Wilderness	Dunged	Unmanured	Wheat	Fallow	Wilderness	Dunged	Unmanured	Wheat	Fallow	Wilderness	Dunged	Unmanured	Wheat	Fallow
June 3, 1913	12	11	7	11	11	6	9	3	3	7	—	—	19	9	4
July 11 „	15	11	8	9	8	7	7	6	4	18	—	21	9	6	11
Aug. 29 „	12	10	5	6	4	4	14	7	—	15	19	14	7	6	8
Sept. 22 „	17	16	13	13	12	6	17	6	3	15	26	30	4	11	12
Oct. 6 „	21	18	15	15	14	3	18	8	3	20	9	29	17	9	11
Oct. 17 „	19	20	15	16	15	5	17	7	14	3	17	24	15	7	15
Nov. 10 „	20	22	17	16	16	4	15	6	8	4	20	30	18	13	8
Nov. 22 „	—	19	16	—	—	—	13	3	—	—	—	—	—	—	—
Dec. 9 „	21	21	17	16	15	15	22	16	16	12	22	35	12	9	8
Dec. 22 „	18	21	16	16	15	9	29	20	14	11	12	30	22	9	9
Jan. 8, 1914	18	23	17	17	17	20	29	17	20	18	13	26	8	7	6
Jan. 20 „	20	27	16	16	16	18	25	18	17	16	14	25	10	14	9
Jan. 30 „	18	21	17	17	17	7	14	9	9	13	13	—	11	11	11
Feb. 12 „	20	24	16	15	17	4	19	3	5	4	—	—	—	—	—
Mar. 2 „	20	21	16	16	16	—	—	—	—	—	—	—	—	—	—
Mar. 11 „	21	22	16	6	7	7	14	7	4	7	—	—	—	—	—
Mar. 31 „	21	22	16	16	16	8	15	9	12	10	—	—	—	—	—
April 30 „	—	16	11	—	—	—	27	24	—	—	—	—	—	—	—
May 11 „	19	18	13	12	13	—	—	—	—	—	12	9	8	8	8
May 18 „	—	12	11	—	—	—	12	12	—	—	—	—	—	—	—
May 25 „	15	13	11	9	11	10	11	10	11	17	12	24	11	7	6
June 10 „	18	18	13	13	12	11	19	12	12	15	—	—	—	—	—
June 12 „	18	19	15	16	15	—	—	—	—	—	—	24	16	14	20
June 26 „	—	13	11	—	—	—	—	—	—	—	—	—	—	—	—
July 7 „	16	17	12	13	13	6	15	15	12	17	29	31	16	15	10
July 21 „	—	14	9	—	—	—	11	9	—	—	—	—	—	—	—
July 27 „	9	9	5	6	7	7	6	5	7	16	—	10	7	6	8
Aug. 13 „	—	14	11	—	—	—	6	7	—	—	—	—	—	—	—
Sept. 12 „	15	15	11	8	10	6	12	9	14	19	—	13	8	6	4
Sept. 21 „	16	16	11	—	—	4	3	8	—	—	—	16	7	—	—

Between Oct. 6 and 17 Hoos Wheat of 1913 becomes Hoos Fallow 1914.

(*Received November 20th,* 1914.)

STUDIES ON SOIL PROTOZOA.

By ANDREW CUNNINGHAM, B.Sc. (Edin.),
Research Scholar in Bacteriology.

(*From the Laboratorium für Bakteriologie am Landwirtschaftlichen Institut der Universität, Leipzig.*)

I. Introduction.

The work discussed in this paper is a continuation of that described in a previous communication to the *Centralblatt für Bakteriologie*[1]. Unfortunately it has been found impossible to bring all the problems taken up to a satisfactory conclusion in the time available. And as, owing to unforeseen circumstances it is necessary, for the present at least, to leave this subject, the results so far as they have been arrived at are brought together in this publication and the lines on which it had been intended to work are indicated. It is hoped, however, that an opportunity of continuing this work on the soil Protozoa may present itself at some future date.

The points which will be dealt with here are:

The dilution method and its application to the enumeration of protozoa in soils.

The effect of protozoa on the numbers of bacteria in ammonifying solutions and on ammonification in solution tests.

The effect of inoculations of protozoa on the bacterial content of partially-sterilised soils.

II. The Dilution Method.

The dilution method has already been applied to the enumeration of protozoa in soils by Rahn[2]. He used as media peptone and sugar solutions incubated for 7–14 days. The dilutions were made in the usual way and at the end of the incubation period the cultures were submitted to microscopic examination. As a result of his work, he found that drying the soil caused a reduction in the numbers of protozoa and that this reduction was first noticeable in the case of the amoebae. Killer[1] also used the dilution method, with a number of the solutions employed for cultivation of soil bacteria as media.

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[1] *Centralbl. f. Bakt.* Abt. II. Bd. **39**, pp. 596–610.

[2] *Ibid.* Bd. **36**, p 419.

The method employed in these experiments is in principle the same as that generally applied in bacteriological work. Four parallels in each dilution are used. The medium is soil extract (prepared as described in Lohnis' *Praktikum*, p. 118, but undiluted) + ·1 % K_2HPO_4 in 1 c.c. quantities in small test-tubes. To each tube 1 c.c. of the dilution-water is added, so that the medium, so far as the protozoa are concerned, is ordinary soil extract + ·05 % K_2HPO_4. But, if the dilutions are put up simply as above described, it has been found that the multiplication of the protozoa after excystation is rather slow and the microscopic work as a consequence is very tedious. It has been observed that inoculation of the soil extract with a protozoa-free culture of bacteria, prepared from a bloodmeal culture as described in the previous paper (p. 604), hastens the multiplication of the protozoa. The microscopic work is thus considerably facilitated. The procedure is to inoculate the soil extract with the protozoa-free culture and incubate for two days before inoculation from the dilutions

Subsequent work on the effects of moisture, etc. on the protozoal content of soils has shown that the dilutions 100, 1000, 10,000, 100 000, etc., are not close enough to bring out differences due to the treatment of the soils. It has, therefore, been found necessary to employ closer dilutions. Those used are, for example, 100, 300, 500, 750, 1000, 3000, 5000, etc.

When now the method is applied to the enumeration of protozoa in soils, it is found that the results are rather irregular. Up to a certain dilution all four parallels in each case give positive results Then in the next three or four dilutions 1–3 of the parallels in each are positive, the remainder negative. Table 1 shows a typical case. The figures given in the columns indicate the number of parallels showing positive results.

It will be observed that after five days' incubation the development is regular up to and including 5000 with single positive results in each of the next four dilutions. On incubation for a further period of 25 days the regular development stage is pushed forward to the 10,000 dilution. But beyond this irregularities still remain From the point of view of regularity, therefore, the 30 days' is no better than the five days' period, although it gives a slightly higher result. But 30 days is as long an incubation period as could be conveniently adopted and consequently it was not considered advisable to investigate the effects of a longer period. And, as five days' incubation gives as satisfactory results as 30 days, the former has been adopted in subsequent work. The question of the higher results obtained after 30 days need not be considered in view of the much greater convenience of the shorter period In any case the dilution method, here as with bacteriological work, gives only relative, not absolute results. The whole of the protozoa in soils do not develop in soil extract.

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[1] *Centralbl f Bakt* Abt. II. Bd 37, p 521.

TABLE 1.

Incubation period (22° C.)	Dilutions					
	5000	7500	10,000	30,000	50,000	75,000
5 days	4	1	1	1	1	0
12 ,,	4	3	3	2	2	0
30 ,,	4	4	4	3	2	2

With a view to obtaining more regularity in the results some slight modifications of the method were tried. In the dilution method, as used in bacteriological work, the addition of small quantities of sterile soil to the medium is found to have a beneficial effect on the growth of the soil bacteria. It was thought that the same might apply to the protozoa but this has not proved to be the case. Indeed, the use of sterile soil results in a much slower excystation of the protozoa and no improvement as regards regularity. The retardation of excystation is probably due to the extraordinarily beneficial effect which the soil has on the growth of the protozoa-free culture before the inoculation with protozoa. It has frequently been observed that the development of protozoa in a medium containing exceptionally large numbers of bacteria is considerably hindered. In a further experiment the inoculation with protozoa-free culture was omitted, only sterile soil being added. The results obtained were very low and quite as irregular as in the previous case.

The effect of the reaction of the medium was next tested. But soil extract + chalk as well as soil extract + ·01 % hydrochloric acid and soil extract + ·01 % caustic potash effected no improvement with regard to the regularity of the results. The ordinary soil extract + ·1 % K_2HPO_4 + protozoa-free culture was therefore used in all subsequent work and the last dilution in which all four parallels gave a positive result after five days' incubation at 22° C. was adopted (quite arbitrarily, of course) as the protozoal content of the material examined. In all cases the results are given as numbers per gram of soil.

With regard to the cause of the irregularity in the development in the dilutions, it is most probably to be explained on the supposition that the protozoa adhere very readily to the soil particles. It is exceedingly likely that the amoebae in particular may be carried over from dilution to dilution in this way.

In the last paper, 58° C. was suggested as a temperature which would kill off all active soil protozoa capable of development on soil extract and so allow of a *distinction* being drawn *between active and encysted forms*. This temperature has been adopted in combination with the dilution method already described. Two sets of dilutions are generally made, the first with the untreated soil, the second with the soil after heating to 58° C. The heating is generally carried out in the 100 dilution.

TABLE 2.

	Dilutions				
Sample A	750	1000	3000	5000	7500
Total Nos	4	4	2	2	2
After heating to 58° .	4	3	1	0	0
Sample B	100	300	500	750	1000
Total Nos.	4	4	4	4	3
After heating to 58° ..	4	4	3	2	2

As a result of further work it appears probable that a temperature of 58° C. kills a number of the encysted protozoa in addition to the active forms. Thus, for example, it has been found that pure cultures of certain flagellate and ciliate cysts do not excyst after being heated to 58° C. and subsequently brought into fresh media. The results of some experiments on the effect of drying on the protozoa may also be cited in this connection. Two samples of soil were allowed to dry at

22° C., A for nine days, B for 16 days. Protozoa counts were then made as above described. Table 2 shows the results.

In these cases as a result of drying one would naturally expect at least a certain number of the protozoa to encyst and the total numbers to be equal to those obtained after heating to 58° C. provided the latter treatment had no injurious action on the cysts. But in both cases the heating appears to have destroyed a number of the encysted organisms. In the first case the distinction is small but in the second it is considerable.

In order to obtain further evidence on this point the effect of treatment with caustic potash on cysts and active forms was examined. As a preliminary experiment soil extract cultures of protozoa showing numerous active and encysted forms were treated for varying lengths of time with equal quantities of a ·5 % caustic potash solution, so that the concentration of the alkali in the cultures was ·25 %. At the end of the period of treatment a drop of phenolphthalein solution was added to each culture and the potash neutralised with dilute lactic acid. The cultures were allowed to settle for half-an-hour and then examined microscopically. After five days' incubation at 22° C. they were examined once more. The results are given in Table 3. + indicates the presence of active organisms.

TABLE 3.

Interval since Neutralisation	Controls (with addition of phenolphthalein)		Potash allowed to act for:					
			1 minute		1 hour		4 hours	
	A	B	A	B	A	B	A	B
Half hour	+	+	−	−	−	−	−	−
Five days	+	+	+	−	+	+	+	+

The treatment with potash killed all active protozoa but left the encysted uninjured and the latter were able to excyst within five days.

The treatment with ·25 % potash for one hour was applied in the dilution method. Dilutions were made from a sample of soil, in the one case after the soil had been heated to 58° C. in the 100 dilution, in the other after it had been treated with ·25 % potash for one hour, also in the 100 dilution. Table 4 shows the results.

Here it will be observed that the heat has had a more drastic action on the cysts than has the potash. It appeared possible that this distinction might be due to the adsorption of some of the potash by the soil. It was found, however, by titration of the remaining alkali with acid that, under the conditions of the above described experiments, only 10–15 % of the potash was put out of action,—a quantity quite insufficient to account for the difference in the results obtained.

TABLE 4.

Soil	Dilutions				
	500	750	1000	3000	5000
(1) Heated to 58° C ..	4	1	2	1	0
(2) Treated with ·25 % KOH for one hour ..	4	4	3	2	3

From these experiments, therefore, it seems highly probable that heating to 58° C. kills a considerable number of the encysted protozoa. But it has been shown that heating to 58° C. is absolutely necessary if one wishes to make sure of killing off all active forms (particularly ciliates). From what has been said it is evident that it is impossible to fix upon a temperature which will destroy all active protozoa in soils and leave the cysts perfectly uninjured. This was only to be expected. In the case of the bacteria the power of resistance to heat of the active forms varies enormously and sometimes even surpasses that of the spores of less resistant species. The same remark would appear to apply to the protozoa. Further, it must be remembered that during a period of excystation or encystation of a particular species it is quite impossible to draw a hard and fast distinction between cyst and active form. And it is obvious that the various transition forms encountered in such cultures must have very varied powers of resistance to heat. Any temperature selected for the purpose of distinguishing active protozoa from cysts must therefore be of an arbitrary nature. And as it is better to select a temperature which will kill all active forms even if it does injure some of the cysts, rather than one which will leave the cysts unharmed and also probably some of the active forms alive, the continued use of 58° C. seems to be justified. This view is supported by the results of experiments which will presently be discussed. It has been found that the numbers obtained by the dilution method after heating to 58° C. (referred to, later, as "Cysts") show variations corresponding with variations in the treatment of the material. The method, therefore, yields useful results which, after all, is the best justification it can have.

The results of the experiments on the effect of heat quoted above probably rather exaggerate its injurious action. In this connection three points must be kept in mind:

(1) It must be remembered that in those cases in which the cysts failed to excyst after heating to 58° C. pure cultures were dealt with. In the results of experiments on the effect of heat on cysts, described in the previous paper, the thermal death point of the most resistant cysts found in soil is given as 72° C. This does not, however, exclude the possibility of the presence of forms with less resistant cysts.

(2) In the experiments on the effect of drying, the desiccation itself may have had an injurious action on the cysts and as a consequence may have rendered them a more easy prey to the injurious influence of the heat.

(3) In the potash experiment, protozoa which had been cultivated on an artificial medium (soil extract) and thus probably rendered less resistant, are dealt with.

III. THE OCCURRENCE AND ACTIVITY OF PROTOZOA IN SOILS AS INDICATED BY THE DILUTION METHOD.

The relative occurrence of the flagellates and amoebae in soil is indicated in Table 5.

TABLE 5.

Dilutions.....	3000				5000				7500				10,000			
Parallels.....	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
F = flagellates A = amoebae	F	F	FA	F	FA	FA	F	F	FA	F	F	F	F	FA	F	F

Dilutions.....	30,000				50,000				75,000				100,000			
Parallels.....	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
F = flagellates A = amoebae	F	F	—	F	F	—	—	—	—	—	—	F	F	—	—	—

The flagellates are seen to occupy first place. The amoebae are rather fewer in number but this may be due to the fact that they are rather sensitive to the presence of members of the other groups and are probably to some extent suppressed by them in the cultures. The ciliates are always present in much smaller numbers. They are rarely seen in dilutions exceeding 100. But the appearance of the various groups in particular dilutions cannot be considered as giving any very sure indication of the relative occurrence of protozoa in soils.

To the question as to whether the protozoa lead an active life in soil, it has been shown that the action of heat combined with the dilution method does not give a definite answer. That question, however, is answered in the affirmative by the results of experiments which will now be discussed.

(*a*) *The Effect of Temperature.*

(i) *On the number of protozoa in soils.* For these experiments some garden soil was passed through a 2 mm. sieve and placed in an ordinary porous flowerpot. The moisture content was determined and adjusted to 70 % of the water-holding capacity of the soil. It was kept at this degree of moistness by watering with boiled water every day during the course of the experiment. The total numbers of protozoa and cysts growing on soil extract were determined by the method described above, immediately after the first adjustment to 70 % W.H.C. The pot was kept in succession for nine days at 5–7° C., for seven days

TABLE 6.

Total Numbers	Dilutions				
	7500	10,000	30,000	50,000	75,000
In original soil	4	4	2	—	—
After 9 days at 5–7° C ..	4	4	3	1	1
After further 7 days at 22° C	4	4	4	3	2
After further 7 days at 30° C	4	4	3	2	0
Cysts	500	750	1000	3000	5000
In original soil	4	4	3	2	—
After 9 days at 5–7° C ..	4	4	4	1	2
After further 7 days at 22° C	4	4	4	2	2
After further 7 days at 30° C	4	4	3	4	3

at 22° C. and for seven days at 30° C., determinations of total numbers of protozoa and cysts being made before each change of temperature. Table 6 shows the results.

It will be noted that after nine days at 5-7° C. practically no change from the original numbers is observed. This is as was to be expected, for the temperature was about the same as that to which the soil had been exposed in the garden and the only change in the conditions was that the soil in the flowerpot had received about 3 % more water than was present in the plot from which it was taken. But after a period of seven days at 22° C. quite a considerable increase in the total number has taken place while the cysts have remained practically stationary. Exposure to a temperature of 30° C. for seven days has caused a fall in the total numbers but a distinct rise in the number of cysts. The fall in the total numbers is readily explained when one bears in mind that certain of the soil protozoa in active form are killed by a temperature of 25° C. Doflein[1] refers to the work of Grosse-Allermann who showed that *Amoeba terricola* (Greef) is killed after a few hours at 25° C. But apart from this 30° C. is evidently too high a temperature to allow of the activity of quite a number of the protozoa in soils as is shown by the increase in the number of cysts. As the result of these experiments, therefore, a temperature in the neighbourhood of 22° C. seems to be the most suitable for the activity of the majority of the soil protozoa.

But although 22° C. is the optimum for the majority of the protozoa in soils, it does not exclude the possibility of the presence of other organisms adapted to higher temperatures. In order to try to throw some light on this point a further experiment was undertaken. The protozoal content of a sample of soil which had been saturated with water and kept for eight days at 22° C. was determined. The soil was then placed in the 30° C. incubator for 38 days, during which time it was kept saturated with water. Determinations of the numbers of protozoa in the soil after eight and 38 days respectively were made. For all three determinations quantities of the soil corresponding to the same dry weight were employed so that the figures in Table 7 are comparable.

A fall in the total numbers of protozoa is observed after eight days at 30° C. as was to be expected from the results given above. But later the organisms which are adapted to the higher temperature show a marked increase in numbers. It is evident, therefore, that soil contains protozoa adapted to a temperature of about 30° C. and that these become active when the conditions are favourable for their growth.

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[1] *Lehrb d Protozoenkunde*, p. 319

TABLE 7.

Total Numbers	Dilutions				
	10,000	30,000	50,000	75,000	100,000
After 8 days at 22° C	4	4	3	2	1
After 8 days at 30° C	4	1	2	2	1
After 38 days at 30° C	4	4	4	2	—
Cysts	300	500	750	1000	3000
After 8 days at 22° C			--	3	2
After 8 days at 30° C	–	4	3	1	2
After 38 days at 30° C	1	2	2	2	0

(ii) *On the kind of protozoa in soils* Observations on cultures (chiefly bloodmeal solutions + K_2HPO_4) of soil protozoa kept at various temperatures have yielded some interesting results. In such cases at temperatures below 8° C. flagellates only have been observed. These appear to multiply much more rapidly at the low temperature than they do at 22° C., for example, and they continue for a much longer period in the active form. In cultures kept at 22° C. flagellates, ciliates and amoebae may all be present. At 30° C. on the other hand the fauna of culture solutions consists practically entirely of ciliates. A few flagellates are sometimes observed at first. At 38° C. few protozoa develop. Only amoebae have been observed. These points are of importance from the point of view of securing pure cultures of the respective groups.

As to the effect of temperature on the kind of organisms leading an active life in soil, little definite information has been obtained. Ciliates (in addition to flagellates) have been observed directly under the microscope in droplets taken from the surface of a saturated soil kept at 30° C. The forms seen belonged to the genus *Balantiophorus*. Such organisms may, therefore, be of importance in sewage and water-logged soils during hot summer weather in temperate climates and also in the rice-fields of tropical countries.

(b) *Effect of Moisture.*

(i) *On the number of protozoa in soils.* In the following experiments the temperature was kept constant at 22° C.

Experiment I. The water content of a sample of garden soil was adjusted to 70 % of its water-holding capacity and a determination of the number of protozoa present was made by means of the dilution method. The sample was divided into three portions. In the first case 10 grams of the soil was placed in a petri dish. The lid was kept raised so as to allow of evaporation of moisture but prevent contamination from the air. The second portion consisted of 30 grams of soil, also in a petri dish. This sample was saturated with water and the lid allowed to remain in position to prevent evaporation as much as possible. The third portion consisted of the remainder of the sample in a flowerpot covered with cotton-wool to minimise evaporation but allowing free access of air. The first portion was allowed to dry for nine days, the second was kept saturated for eight days, while the third was kept at 70 % W.H.C. for seven days. At the end of these periods a determination of protozoa was made for each portion. In the case of the dry and saturated samples quantities corresponding to one gram of the 70 % sample were taken for the dilutions. The results are shown in Table 8.

TABLE 8.

Total Numbers in	Dilutions						
	1000	3000	5000	7500	10,000	30,000	50,000
Original sample ..	4	4	4	4	4	2	—
Dried sample ..	4	2	2	2	0	1	–
70 % W H C sample	4	4	4	4	4	4	3
Saturated sample	4	4	4	4	4	4	3
Cysts in	**300**	**500**	**750**	**1000**	**3000**	**5000**	**7500**
Original sample ..	4	4	4	3	2	—	—
Dried sample ..	—	—	4	3	1	0	0
70 % W H C sample	4	4	4	4	2	2	1
Saturated sample	—	—	—	3	2	0	1

The effect of drying is seen in the reduction of the total numbers. The 70 % and saturated samples have given the same increase. The cysts show practically no change as a result of the variations in the treatment.

Experiment II. In this case the original sample was divided into two portions. One was allowed to dry for nine days: the other was kept saturated for seven days. Bacterial counts were also made on the samples, agar at 22° C. being used as medium. Otherwise the procedure was the same as in Experiment I. Table 9 shows the results.

TABLE 9.

	Dilutions						Bacteria (millions per gram)
Total Numbers in	750	1000	3000	5000	7500	10,000	
Original sample ..	—	—		4	2	2	13·95
Dried sample ..	4	3	2	2	—	—	6·90
Saturated sample	-	—	4	4	3	3	5·20
Cysts in	100	300	500	750	1000	3000	
Original sample ..	—	—	4	1	2	1	—
Dried sample ..	—	3	2	3	1	0	—
Saturated sample	—	1	1	2	2	0	-

Drying has again resulted in a reduction in the number of protozoa while the saturation of the soil with water has produced a slight increase in total numbers and a very decided decrease in the number of cysts. The bacterial content has in both cases fallen considerably and it is noteworthy that from the saturated soil more bacteria have disappeared than from that which has been exposed to drying. But the conditions in the saturated soil cannot be regarded as very unfavourable for bacterial growth, for the layer of soil and water is quite a thin one (about ¼ inch).

Experiment III. The plan in this case was similar to that adopted in Experiment I, but bacterial counts on agar at 22° were added. The dried soil was kept for 16 days· the 70 % sample for 15 days and the saturated sample for 14 days. Table 10 contains the results.

Here the drying has caused no decrease in the total numbers of protozoa. The latter appear all to have been able to encyst before the soil became too dry for active life. This view is supported by the

TABLE 10.

Total Numbers in	Dilutions						Bacteria (millions per gram)
	750	1000	3000	5000	7500	10,000	
Original sample	4	3	2	1	1	0	8·1
Dried sample	4	3	—	—	—	—	3·1
70 % W.H.C. sample	4	4	4	3	1	2	6·4
Saturated sample	4	4	4	4	4	4	6 7
Cysts in	50	100	300	500	750	1000	
Original sample	0	1	0	0	0	0	—
Dried sample	4	4	4	3	2	2	—
70 % W.H.C. sample	3	2	0	0	0	0	—
Saturated sample	3	1	1	0	0	0	—

fact that the cysts have shown a marked increase in the dried sample. Very decided increases in total numbers are observed in the 70 % and saturated samples, especially the latter. The fall in the bacterial content of the 70 % and saturated samples is not so marked in this instance as it was in the case of the saturated sample in Experiment II. This is probably due to the fact that the protozoal activity had reached its maximum before the counts were made as is indicated by the increase in the number of cysts The same cause has probably resulted in an obliteration of any difference, which might have been expected, in the bacterial contents of the 70 % and saturated samples as a result of the difference in the protozoal content of the latter.

Experiment IV. The 70 % sample, after use in Experiment III, was employed as the starting point in this experiment. The samples to be dried and saturated respectively were taken from it and the remainder was kept for a period of 12 days at 70 % W.H.C. The dried sample was kept for 14 days, the saturated sample for 12 days, and bacterial and protozoal counts were made for all three samples as in the last experiment. The results obtained are given in Table 11

Drying has, in this instance, lowered the numbers of protozoa present, while the cysts again remain considerably behind the total numbers. The 70 % sample is, at the end of the experiment, in practically the same condition as it was at the beginning. It is obvious, therefore, that the protozoa in the sample had reached the maximum of their activity during the course of the preceding experiment. Thus, these results confirm those of Experiment III. The saturated sample in Experiment IV has again shown a great increase in the numbers of protozoa.

TABLE 11.

Total Numbers in	Dilutions									Bacteria
	500	750	1000	3000	5000	7500	10,000	30,000	50,000	
Original sample	—	4	4	4	3	1	2	—	—	6·4
Dried sample	4	3	1	2	—	—	—	—	—	3·8
70 % W.H.C. sample ..	—	—	4	3	3	1	2	3	—	8·0
Saturat'd s'mple	—	—	—	4	4	4	4	4	4	7·2
Cysts in	50	100	300	500	750	1000	3000			
Original sample	3	2	0	0	0	0	0	—	—	—
Dried sample	—	3	1	1	0	0	0	—	—	—
70 % W.H.C. sample ..	2	3	1	0	—	—		—	—	—
Saturat'd s'mple	2	3	1	0	–	–	—	—	—	—

The increase in the bacterial content of the saturated sample as compared with the bacterial content of the 70 % sample at the beginning of the experiment is again probably due to the protozoal activity having passed its maximum. The increase in the bacterial numbers in the 70 % sample during the course of the experiment was only to be expected from what has already been deduced.

(ii) *On the kind of protozoa in soils.* In the cultures made from the dilutions, considerable variations are to be observed in the kind of organisms obtained from saturated, 70 % W.H.C. and dry soils. In cultures from saturated soils practically only flagellates are found. The 70 % and dried samples on the other hand yield amoebae in addition to flagellates. Ciliates are seldom seen in any of the cultures. It seems highly probable, therefore, that the flagellates may require a rather moist medium for the unfolding of their activities. The amoebae appear to prefer a somewhat drier soil. But it is possible that they may also lead an active life in saturated soils but may be suppressed in the cultures by the flagellates which are present in large numbers in such soils.

To summarise the results of these experiments on the effects of temperature and moisture on the soil protozoa:—*It has been shown that some, at least, of the protozoa in soils lead an active life and are capable of multiplying to quite a considerable extent when the conditions become favourable. It is also very probable that those protozoa which do lead an active life in soils (as indicated by the dilution method) are capable of limiting the numbers of bacteria present in the latter.* But this point still requires some elucidation.

IV. THE INFLUENCE OF PROTOZOA ON THE NUMBERS OF BACTERIA DEVELOPING IN AMMONIFYING SOLUTIONS.

In order to obtain some information on the capacity of soil protozoa for destroying bacteria in solutions, it was thought necessary to have a method of suppressing the former. In the literature one finds that P. T. Müller[1] employed Saponin for this purpose, in connection with his investigations on the protozoa of swimming-baths. The concentration used was ·5 % and it is stated that this had no injurious action on the bacteria.

The use of Saponin was, therefore, applied to ammonifying solutions inoculated with soil. 1 % bloodmeal in water was heated to one and a half atmospheres in the autoclave and filtered. ·05 % K_2HPO_4 was added and 100 c.c. of the solution sterilised. After cooling this nutrient solution was inoculated with 5 grams of garden soil and incubated for 18 hours at 22° C. The bacterial content of the solution was determined (agar, incubated at 22° C., has been used as medium for bacterial counts all through this section and the results are stated in the tables as millions per c.c.). The solution was divided into two equal parts in small sterile flasks and one portion received ·5 % saponin. Plates were poured from both portions at the intervals indicated in Table 12.

The active protozoa present were counted by the microscopic method. The immediate effect of the saponin is seen in the depression in the numbers of bacteria in the solution. This, however, does not last long. After 24 hours the protozoa developing in the solution without saponin begin to exercise a decided depressing effect on the number of bacteria and this has continued throughout the experiment. But the contrast between the bacterial contents of the two portions is doubtless somewhat minimised because the saponin has failed to suppress entirely the protozoa.

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[1] *Arch f Hyg.* Bd. 75, 1912, p. 321.

TABLE 12. *Bacterial Content of solution before division* = 2·6.

Time since division of solution	Solution with saponin		Solution without saponin	
	Bacteria (c c)	Protozoa (c c)	Bacteria (c c)	Protozoa (c c)
1 hour	4·55	—	6·45	—
3 hours	9·05	—	12·80	—
6 "	12·90	—	31·00	—
24 "	75·00	—	56·00	400 F
4 days	100·00	400 F	56·00	15,000 F 1000 C
10 "	68·00	200 F and C	26·00	200 F 1200 C
20 "	220·00	1200 C 400 A	23·50	Under 200
30 "	260·00	—	14·90	—

F = flagellates C = ciliates A = amoebae.

After 10 days a clearing in the saponin solution set in which, taken in conjunction with the great increase in the bacterial content, appears to point to the decomposition of the saponin. In order to test this two equal quantities of filtered 1 % bloodmeal solution + K_2HPO_4, one of which contained ·5 % saponin, were each inoculated with 1 c.c. of a protozoa-free culture of bacteria Bacterial counts were made at the intervals shown in Table 13.

TABLE 13.

Time since inoculation of solutions	Bacterial content of solution	
	With Saponin	Without Saponin
1 day	185	185
4 days	1250	1700
10 "	2300	1050
20 "	1600	650

From the results here obtained it is very probable that the bacteria attack the saponin and that the resulting increase in bacterial numbers will exaggerate the destructive effect of the protozoa. A second disadvantage in the use of saponin is that at a concentration of ·5 % it does not entirely suppress the protozoa. Higher concentrations have been tried but up to 3 % one can never be certain that the whole of the protozoa will be excluded. It appears, therefore, that saponin is of little value for this purpose and its use has been abandoned. In the work described by P. T. Müller the action of the saponin was quite satisfactory. But it must be noted that water was employed as the medium, not a nutrient solution

Recourse was next had to the simple method of inoculation of the solutions with bacteria alone and with bacteria + protozoa. 50 c.c. quantities of 1 % bloodmeal solution (filtered) + ·05 % K_2HPO_4 were employed. One flask was inoculated with bacteria + protozoa from a culture of protozoa from soil, the other received as nearly as possible an equal inoculation from the same culture of bacteria alone. The method of inoculation was the single drop method already referred to. Table 14 shows the numbers of bacteria and protozoa developing in the solution.

TABLE 14.

Time since inoculation of solution (days)	A			B		
	Bacteria alone	Bacteria + Protozoa		Bacteria alone	Bacteria + Protozoa	
		Bacteria	Protozoa		Bacteria	Protozoa
1	10	8	—	Fewer than ·01	·03	—
6	736	505	65,000 F	860	801	—
10	625	350	25,000 F	2100	1400	C under 200
20	700	270	15,000 F	1120	49	1600 C
30	370	53	25,000 F	635	21	200 C

It will be observed that in both experiments the solutions to be compared started with practically equal inoculations of bacteria and that the subsequent depression in the bacterial numbers is marked and runs more or less parallel with the numbers of active protozoa present. In Experiment B, after 20 days in addition to the 1600 ciliates given at least 50,000 cysts were counted. This accounts for the very rapid fall in the number of bacteria between the tenth and twentieth days. The results after 30 days indicate very clearly the destructive power of the protozoa. In A, flagellates only were present; in B ciliates only, and as was to be expected the results show that the latter are the more active in the killing off of the bacteria.

This method of inoculation, although it has given quite good results, is not entirely satisfactory. The difficulty lies in the uncertainty as to whether the protozoa will develop after inoculation. This is probably due to the fact that the inoculum is very small compared with the bulk of the medium. The protozoa are thus forced to encyst until the bacteria develop and during this process the bacteria very frequently appear to take the upper hand.

Another method of inoculation was tried. The medium (·4 % bloodmeal, unfiltered, + ·05 % K_2HPO_4 in 100 c.c. quantities in Erlenmeyer flasks) was inoculated from a protozoa-free bloodmeal culture, each flask receiving a loopful. After two days at 22° C. some of the flasks received in addition a loopful of a bloodmeal culture containing protozoa from soil, so that from the beginning they contained more bacteria than the protozoa-free cultures. The development of the protozoa was now much more regular. Bacterial counts were made after 10 and 20 days and the numbers of active protozoa were determined roughly by the microscopic method. The results are shown in Table 15.

TABLE 15.

No of Expt	After 10 days			After 20 days		
	Bacteria alone	Bacteria + Protozoa		Bacteria alone	Bacteria + Protozoa	
		Bacteria	Protozoa		Bacteria	Protozoa
1	480	260	30,000 F	167	156	10,000 F
2	790	420	5,000 F	260	358	0
3	530	440	5,000 F	510	235	All encysted
4		600	10,000 F		320	All encysted
5	870	480	25,000 F	420	90	60,000 F
6		780	20,000 A		150	All encysted

Quite a marked reduction in the bacterial numbers is obtained as a result of the presence of the protozoa in all six experiments. The reduction is, however, somewhat variable and even varies during the course of the individual experiments. In 2, for example, although the protozoa have caused a great reduction in the numbers of bacteria after 10 days, after 20 days the number of bacteria in the protozoa culture is actually higher than in the protozoa-free culture. The protozoa present in this case were large flagellates. But after 20 days no traces of protozoa, active or encysted, could be found. The protozoa had probably died off without encysting and then been attacked by the bacteria. This view receives support from the frequent observation in ammonifying solutions of protozoa, showing absolutely no signs of life but yet without any traces of a cyst membrane surrounding them. It is quite probable, therefore, that some species of protozoa die off without being able to encyst when the concentration of ammonia or other products of the activity of bacteria reaches a particular level. Their bodies would then be a ready prey to the attacks of bacteria and the latter might increase in numbers as a consequence.

The reductions in the numbers of bacteria as obtained in these experiments are on the average smaller than those given in Table 14. But it must be remembered that the bacterial content of the protozoa cultures at the beginning was in all cases larger, probably often much larger, than that of the protozoa-free culture. The only satisfactory method for securing comparable results, therefore, is the inoculation of equal numbers of bacteria from a protozoa culture in the one instance and from a protozoa-free culture (prepared from the protozoa culture) in the other, on to fresh media and the determination of bacterial numbers in both solutions at intervals.

The results given in this section prove conclusively that the soil protozoa, in solutions at all events, exercise a very decided limiting effect on the numbers of bacteria. The question of the relative activity in this direction of the three main groups of protozoa—flagellates, ciliates and amoebae—remains to be investigated.

V. THE INFLUENCE OF PROTOZOA ON AMMONIFICATION IN SOLUTION TESTS.

As a preliminary experiment in this direction, the quantities of ammonia produced in some of the cultures used in the last section were determined. The conditions in these cultures may be briefly recapitulated. Each culture contained ·4 grm. bloodmeal + ·05 grm. K_2HPO_4 in 100 c.c. water. After sterilisation in the autoclave at two atmospheres pressure, each was inoculated with one loopful of a protozoa-free bloodmeal culture and incubated for two days at 22° C. Some of the cultures then received each one loopful of a bloodmeal protozoa culture from soil. At the end of the incubation period (20 days at 22° C.) all were distilled with magnesia and the ammonia evolved determined (see Table 16, which gives the results after deduction of controls).

TABLE 16.

Number of Experiment	Mgs nitrogen as ammonia in culture containing:—	
	Bacteria alone	Bacteria + Protozoa
1	21·4	21·3
2	20·6	19·4
3	19·6	17·5
4		18·3
5	19·7	18·0
6		19·0

From the results of the bacterial counts (Table 15) one would naturally expect that ammonification would be depressed in presence of the protozoa. But the protozoa cultures have given an ammonification figure only slightly lower than that obtained in the protozoa-free cultures. The difference is comparatively insignificant. When the conditions prevailing in these experiments are kept in mind it seems probable that the higher original bacterial content of the protozoa cultures may account for the unexpectedly high ammonification number obtained from them. It is probable that the ammonification in the protozoa cultures, before development of the latter organisms, may have been very rapid— so rapid that the subsequent fall in bacterial numbers and consequent ammonifying power has been only just capable of neutralising it.

The only satisfactory method of deciding the matter seemed to be the inoculation of equal numbers of bacteria into solutions with and without protozoa. The microscopic method of counting bacteria was employed for this purpose. But in the case of these bloodmeal solutions the method was rather uncertain in its results, because of the difficulty in distinguishing the smaller species of bacteria from fine particles of bloodmeal, etc. The numbers of bacteria counted in the solutions, as a result of plating on agar, showed wide differences from those given by the microscopic method. In the first set of experiments the solutions were inoculated from bloodmeal cultures of protozoa + bacteria and bacteria alone, respectively. The inoculations of bacteria were arranged by the microscopic counting method so as to be approximately equal. The counts on agar at 22° C. indicated, however, that the protozoa-free cultures had each received about 353 millions, the protozoa cultures on the other hand 440 millions of bacteria. The solutions employed were similar to those used in the last experiment. It was found advantageous to incubate all the cultures for two days with equal inoculations of protozoa-free culture before inoculation with bacteria or bacteria + protozoa as the case might be. The solutions were incubated for a total period of 20 days, from the first inoculation, at 22° C. The protozoa were present in observable numbers in two days after inoculation—*i.e.* four days from the first inoculation with bacteria. The ammonia was determined by distillation with magnesia and the results so obtained (after deduction of controls) are shown in Table 17.

TABLE 17.

Number of Experiment	Mgs nitrogen as ammonia in solution containing:—	
	Bacteria alone	Bacteria + Protozoa
1		15·3
2	19·3	16·4
3	21·2	14·3
4		15·2
5		15·7

In spite of the fact that the protozoa cultures started out with an inoculation of 87 millions or about ¼ more bacteria than the protozoa-free cultures, they give a markedly lower figure for ammonification. The averages are, for the protozoa-free cultures 20·3 mg N. and for the protozoa cultures 15·4 mg. N. This difference lies well outside the limits of experimental error.

In the last experiment which it has been possible to carry out in this direction, the bloodmeal cultures were inoculated, as above described, with 580 millions bacteria and 480 millions bacteria + protozoa (as indicated by counts on agar at 22° C.). The conditions were otherwise the same as in the previous experiment. The quantities of ammonia produced in the cultures after 20 days at 22° C. are shown in Table 18. (Controls have been deducted.)

It is unfortunate that in this case the original inoculation of bacteria in the bacteria + protozoa cultures was so much smaller than that in the bacterial cultures. The experiment is, therefore, of little value in helping towards a solution of the question.

TABLE 18.

Number of Experiment	Mgs. nitrogen as ammonia in solution containing:—	
	Bacteria alone	Bacteria + Protozoa
		16·2
	19·5	17·3
	19·6	16·9
		16·0

As to the appearance of the cultures with and without protozoa the latter have generally been somewhat brown in colour, the former greenish. Further the two sets of solutions smell quite differently. In the protozoa cultures the vile-smelling decomposition products usually associated with ammonification appear to be absent.

It had been intended to carry this section of the work much further but circumstances unfortunately do not permit. The results, so far as obtained, do not justify any very definite conclusions. The organisms dealt with are, with one exception, the flagellates, and it seems probable that these may have a depressing influence on ammonification. The whole question, however, requires to be thoroughly investigated.

VI THE INOCULATION OF PROTOZOA INTO PARTIALLY STERILISED SOILS.

In the second paper of Russell and Hutchinson[1] on the effect of partial sterilisation of soils, it is stated that the authors have failed to observe a depression in the numbers of bacteria in partially sterilised soils as a result of inoculation with mass cultures of protozoa. This is attributed to the great multiplication of bacteria which takes place on the introduction of the considerable quantity of nutrient material contained in the culture. Greig Smith[2] also failed to obtain a reduction in the numbers of bacteria, after inoculation of partially sterilised soil with protozoa cultures.

Two experiments bearing on this point have been carried out here. For the first experiment 500 grams of air-dry soil was passed through a 2 mm. sieve. 2·5 c.c. formalin in 20 c.c. water was rubbed up with the soil in a mortar and allowed to act in a glass bottle with close-fitting stopper for six days. A sterile suspension of 3 grams freshly slaked lime in 50 c.c. water was added to combine with the formalin and render it harmless. The bottle was placed in the 38° C. incubator for one day. After some weeks at room temperature the soil was thoroughly broken up with a large, sterile, metal spatula and weighed out in 20 gram quantities into sterile Erlenmeyer flasks. The water content was not determined but it probably amounted to about 10 %.

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[1] *Journ of Agric Sc* v 2, p. 152

[2] *Proc Linn Soc. N S Wales,* Abstracts, 1912, pp. 2–3; Ref. *Centralbl f. Bakt* Abt II Bd **39**, p 152

In order to try to minimise, as much as possible, the effects of the nutrient matter in the protozoa culture solution, soil extract + ·05 % K_2HPO_4 was selected as medium. This was inoculated with soil and after the protozoa had developed a protozoa-free culture was prepared from it. Both soil extract cultures were kept for about two months before being used for inoculation purposes. Two of the flasks containing sterilised soil received each 1 c.c. of the protozoa culture, the other two 1 c.c. of protozoa-free culture. All four received 1 c.c. of sterile water each, in addition, in order to bring up the water content of the soil to about 20 % (roughly 70 % of the water-holding capacity). In order to represent, more or less, the conditions obtaining in Russell and Hutchinson's experiments a second series of four flasks was inoculated, two with protozoa + bacteria and two with bacteria alone as in the last case. The sterile water was replaced in this instance by an equal quantity of a sterile 2 % filtered fleshmeal solution. Of the controls two received 2 c.c. sterile water each, the remaining two each 1 c.c. sterile water and 1 c.c. sterile fleshmeal solution.

The bacterial content of the protozoa-free culture was 121 millions per c.c.: that of the protozoa culture 12 millions per c.c. (agar at 22° C. was used as medium for the counts in this section). The numbers of bacteria in the soil samples used in the experiment were determined after 20 days at 22° C. The water contents were adjusted once more to roughly 20 % with sterile water and the flasks were allowed to remain for a further period of 20 days at 22° C. The bacterial contents of the soil samples were again determined (Table 19).

The results of the bacterial counts are rather irregular. This is probably due to the fact that the soil samples used were only watered once during the experiment. The inoculation of bacteria, therefore, probably did not get thoroughly distributed in the soil. The only cultures which have shown a decided depression in bacterial numbers after 40 days (as compared with 20) are Nos. 7 and 8. Here the lowering in numbers is quite marked and considerably larger than in any other case. After the bacterial counts were made the soil samples were covered with soil extract + K_2HPO_4 and incubated for seven days at 22° C. At the end of this period the cultures so made from Nos. 3, 4, 7 and 8 contained active protozoa. Nos. 7 and 8 showed decidedly larger numbers than did 3 and 4. The remaining four soil samples as well as the controls showed no protozoa. But the original "sterilised" soil and the controls contained numerous bacteria.

TABLE 19.

No.	Inoculation	Bacterial content (millions per gram) after	
		20 days	40 days
1	1 c.c. protozoa-free culture + 1 c.c. sterile water	155	100
2		240	240
3	1 c.c. protozoa culture + 1 c.c. sterile water	180	200
4		110	160
5	1 c.c. protozoa-free culture + 1 c.c. sterile fleshmeal soln.	170	250
6		255	220
7	1 c.c. protozoa culture + 1 c.c. sterile fleshmeal soln.	310	200
8		340	140

From the results here given it is probable that the inoculated protozoa have been active in Nos. 7 and 8. But the period of activity under the conditions of the experiment must have been a short one, as after the single watering the soil would very soon become too dry for active life. This, in all probability, accounts for the comparatively small depression in bacterial numbers.

For the confirmatory experiment the soil was sterilised with formalin in the flasks in which it was to be subsequently used. Quantities of 50 grams of air-dry sieved soil were rubbed up in a mortar with 2 c.c. of a solution containing 5 c.c. formalin + 35 c.c. water. Forty-five grams of the soil was immediately weighed out into each of the flasks. The flasks used were small Erlenmeyers closed by tight-fitting corks. The formalin was allowed to act for six days and was then decomposed with slaked lime as described in the last experiment. Each flask received 5 c.c. of a sterile suspension of 5 grams $Ca(OH)_2$ in 100 c.c. water (water content of soil in flasks = 70 % W.H.C.). The flasks were placed in the 38° C. incubator for 24 hours. The soil in each was thoroughly broken up with a sterile spatula and the flasks put back in the incubator for another day. The corks were then replaced by sterile cotton-wool stoppers and the flasks weighed. After several days in the 38° C. incubator to hasten evaporation, the flasks received the inoculations shown in Table 20 and the water content of the soil was brought up to 70 % W.H.C. The water content was readjusted once a week to this level and after 25 days bacterial counts were made for the various soil samples.

TABLE 20.

No.	Inoculation	Bacterial Content (millions per gram)
1	1 c.c. protozoa-free culture	100
2	+ 1 c.c. protozoa culture + 1 c.c. sterile water	52
3	1 c.c. protozoa-free culture	133
4	+ 1 c.c. protozoa culture + 1 c.c sterile 2 % fleshmeal solution	77
5	1 c.c. protozoa-free culture	—
6	+ 2 c.c. sterile water	860
7	1 c.c. protozoa-free culture	420
8	+ 1 c.c. sterile water + 1 c.c. sterile fleshmeal solution	950
9	2 c.c. sterile water + 1 c.c. sterile fleshmeal solution	—
10	3 c.c. sterile water	—

Soil extract cultures were prepared from the soil samples as in the last experiment. Those from Nos. 1–4 showed numerous active flagellates after seven days at 22° C. In the remainder of the cultures no protozoa were found. The controls 9 and 10 remained practically sterile. They contained fewer than 10 bacteria per gram. The plates poured for No. 5 remained sterile. The lowest dilution used was one million. It is practically certain, however, that this must have been due to a slip in the manipulation, and as the samples had been used for soil extract cultures before it was discovered, the mistake could not be rectified. At all events the soil extract culture showed quite as good a development of bacteria as was got from samples 6, 7 and 8.

The protozoa-free culture contained 184 millions, the protozoa culture 24 millions bacteria per c.c. and as the soils inoculated with protozoa received in addition 1 c.c. of the protozoa-free culture they contained at the beginning of the experiment about 24 millions more bacteria than the soils inoculated with protozoa-free culture alone. But during the course of the experiment the conditions have become reversed and the soils containing protozoa now show a bacterial content of, on the average, about ⅛ that of the soils inoculated with protozoa-free culture. *The reduction in bacterial numbers in the soils inoculated* *with protozoa is very marked and lies well outside the limits of experimental error.* The conclusion may safely be drawn, therefore, that the limiting factor or at least one limiting factor (of Russell and Hutchinson) has been inoculated into the sterilised soils and has produced its effects on the numbers of bacteria. This limiting factor can thus be cultivated on soil extract medium. That it has not simply been introduced into the sterilised soils with the soil used for inoculation of the soil extract (*i.e.* without having grown on the latter) is proved by the fact that for the second experiment sub-cultures (made by inoculation of one loopful of the original cultures on to fresh sterile medium) were used. Large numbers of protozoa were observed in the solutions used for inoculation and these organisms were cultivated once more on soil extract from the soils which showed low bacterial counts. And, as it has been shown that the protozoa are capable of reducing the numbers of bacteria in solutions, it appears justifiable to consider them as the limiting factor in soils.

In conclusion I wish to thank Prof. Löhnis for having suggested this work on the soil protozoa and for advice, ever at my disposal, during the carrying out of it.

[This paper was published in the *Centralbl. f. Bakt.* Abt. II. of August last, but owing to the war no copies of it have yet reached this country.]

(*Received December 21st*, 1914.)

STUDIES ON THE LIME REQUIREMENTS OF CERTAIN SOILS.

By HENRY BROUGHAM HUTCHINSON
AND KENNETH MACLENNAN
(*Carnegie Research Fellow*).

(*Lawes Agricultural Trust, Rothamsted Experimental Station.*)

(With Plate I and 4 text-figures.)

THE fertility of a soil may be stated to depend on two functions, namely, its capacity to supply the necessary nutrients for plant growth, either by virtue of original reserves or by biological action, and its suitability as a matrix, serving to hold plant roots and possessing definite relations to air, water and temperature; these determine very largely the amount of growth attained by any crop. Since, however, the growth of soil organisms as well as that of any introduced plants is very sensitive to the reaction of the matrix, it follows that cases may arise in which the presence or absence of a base would definitely set a limit on crop production.

The compounds commonly used to correct soil reaction in the field are those of calcium—either as oxide, hydroxide, or carbonate, as in chalk, limestone, or marl; the benefits accruing from their use are well recognised, but the difference in their type of action has not hitherto received the attention it deserves.

In two earlier communications it has been shown that caustic lime exercises a specific effect on the soil, producing when applied in sufficient quantity certain effects common to those which have been classed under the head of "partial sterilisation." Calcium carbonate does not exercise this action, but either form may be used for correcting soil reaction. In practice this difference has not been recognised and has been responsible in many instances for the misinterpretation of experimental work, since it is difficult to determine precisely how far any result has been due to neutralisation or to sterilisation effects.

It is necessary therefore in any particular case to decide firstly on the type of change to be induced (sterilisation or neutralisation), and secondly, the amount of lime required to bring about the desired effect. The first consideration can only be decided by reference to the particular conditions of each case, while guidance as to the second is afforded by the two methods suggested below for the two distinct purposes. We propose accordingly to divide the paper into two sections, viz.,

Part I. The Determination of the Amount of Lime (CaO) necessary to induce Partial Sterilisation Changes.

Part II. The Determination of the Amount of Lime (CaO) or Chalk ($CaCO_3$) required for Soil Neutralisation[1].

PART I. LIME REQUIREMENTS FOR PARTIAL STERILISATION PURPOSES.

The capacity of caustic lime to induce typical partial sterilisation changes has been studied with a number of soils and the results of these bacteriological, chemical and pot-culture observations have already been given in detail elsewhere (5). The results thus obtained serve as a check on those given by the method below.

The addition to the soil of any partial sterilisation agent results in changes of which the three chief are (*a*) an initial reduction in the numbers of putrefactive bacteria, with a subsequent increase in numbers and activity; (*b*) the partial or complete inhibition of nitrifying organisms, thus leading to accumulation of the ammonia produced by break-down processes, and (*c*) a similar inhibition of the larger soil protozoa With the two latter, somewhat related effects on "soil pests" might possibly be associated.

During this work it was evident that the various soils differed greatly in response to treatment and that the conventional methods failed to give any index to this relative responsiveness. Since the desired changes, chiefly of a biological nature, could only be due to an alteration in the reaction of the soil solution, it was obviously necessary to obtain some gauge of the absorptive power of the soil for lime.

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[1] This term is used with due reservation. Although the view has been advanced by van Bemmelen (1) and Baumann and Gully (2) that the phenomena of "soil acidity" may be explained on a purely physical basis, this has been severely criticised by Rindell (3) and Tacke and Süchting (4) and the authors consider that it would be somewhat premature to abandon the term "acidity" It is therefore used in these pages as convenient to indicate "apparent acidity," "lack of basicity," or in this case "lime requirement"; "neutralisation" may be interpreted as the correction of this condition.

Some observations on this question were made by Way (6), who employed lime water, and further work might naturally proceed along these lines, or by adding solid calcium oxide in varying quantities to the moist soil to be tested. The latter method promised, however, to approach more closely to the conditions obtaining in our other bacteriological and chemical experiments with soil in bottles, and was accordingly adopted. We confined ourselves in the first instance to the study of some five soils—Rothamsted, Millbrook, Woburn, Chelsea and Craibstone—which had been studied in other directions.

The Method. The method originally adopted and to which we have adhered throughout is based on the determination of the minimum amount of lime required to render the soil water distinctly alkaline and is as follows: 100 grm. lots of the air-dry soil to be tested are placed in bottles of about 250 c.c. capacity; according to the character of the soil (whether poor or rich, light or heavy) a number of dressings of calcium oxide are then made, rising by increments of 0·1 grm. to 1·0 per cent., or increments of 0·2 grm. to 2·0 per cent. of the weight of soil. Sufficient water (50 c c.) is added to moisten the soil, the bottles are then tightly corked and shaken for a few seconds at intervals for a definite period. This period is generally 24 hours, but actual comparisons have shown that the amount of change between 4 and 24 hours is only slight. At the end of this time the contents of the bottles are then transferred to, and washed in, a Buchner funnel with a further 200 c.c. of water; the whole of the filtrate is then titrated with N/10 acid, using phenolphthalein as indicator.

Within the range of applications made in the above manner it will generally be found that a point is reached where the reaction of the filtrate is distinctly alkaline, and the results of other investigations have shown that where the alkalinity is such that 5–10 c.c. of N/10 acid are required to neutralise the whole of the filtrate, this may be taken as the limit to which calcium oxide must be applied to the soil in order to produce the best results.

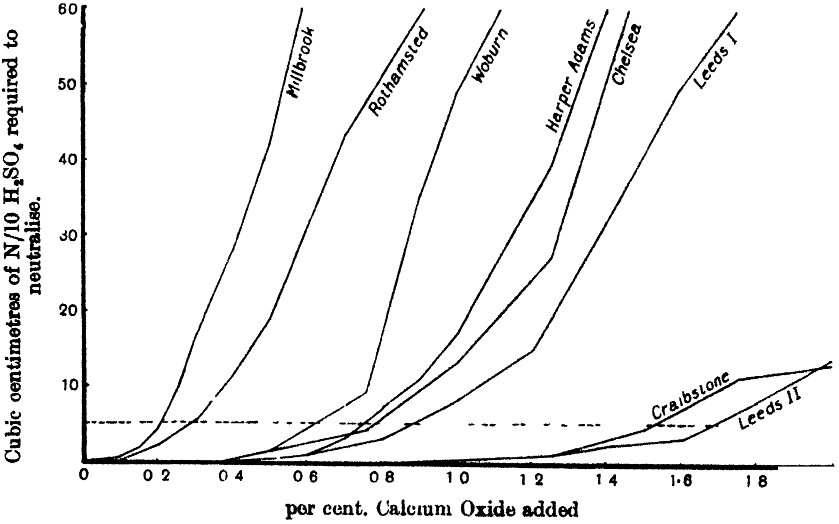
With heavier applications the concentration of the filtrate tends to approach saturation point, but any increase in the application beyond the amount necessary to bring the filtrate to the above-mentioned point appears undesirable; such applications tend to constitute "over-liming" if made under ordinary conditions. The results of a few such tests are given in Table I, and are plotted, together with data obtained with other soils, in Curve 1.

The great difference in absorptive capacity of the various soils is apparent, while the order in which these are ranged possesses certain points of interest that may be noted here.

TABLE I. *The Determination of the Partial Sterilisation Point by the Titration Method.*

(The results are expressed as c.c. N/10 acid required to neutralise the whole of the filtrate.)

Rothamsted		Millbrook		Woburn		Chelsea		Craibstone	
CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.	CaO added	Titrn. c.c.
%		%		%		%		%	
0·1	0·5	0·1	0·5	0·50	2·0	0·25	0·9	0·25	0
0·2	2·2	0·2	4·5	0·75	9·5	0·50	1·8	0·50	0
0·3	5·6	0·3	16·6	0·90	35·0	0·75	4·6	0·75	0·1
0·4	11·5	0·4	27·6	1·00	48·9	1·00	13·4	1·00	0·6
0·5	18·2	0·5	42·1	1·25	75·4	1·25	27·2	1·25	1·2
0·6	26·9	0·6	63·5	1·50	84·2	1·50	66·8	1·50	4·6
1·0	67·3	1·0	95·3	2·00	93·0	2·00	88·9	2·00	12·8



Curve 1. The Determination of the Amount of Caustic Lime required to induce Partial Sterilisation of a Soil.

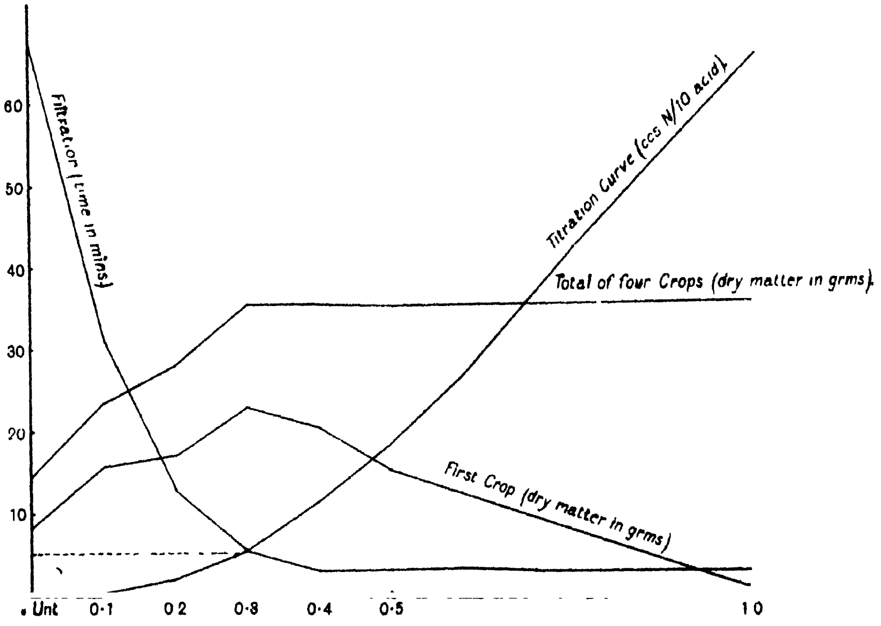
The addition to any soil of the amount of lime indicated by this titration method is sufficient to affect appreciably the reaction of the soil water and hence may be expected to displace more or less radically the biological conditions obtaining therein. This we find to be the case with all the soils over which an adequate control has been exercised. For purposes of comparison the relations of the nitrifying organisms and the larger soil protozoa towards treatment are given in the following table.

Table II. *The Relation between the Critical Point indicated by Titration Method and the Amounts of Lime necessary to induce certain changes in various Soils.*

	Rothamsted	Millbrook	Woburn	Chelsea	Craibstone
Critical point indicated by titration method	0·3	0·2	0·6	0·75	1·0
Inhibition of nitrification (laboratory experiment)	0·3	0·3	0·5–1·0	0·5–1·0	1·0
Destruction of larger protozoa (laboratory experiment)	0·3–0·4	0·2–0·3	0·5–1·0	0·5–1·0	1·0
Maximum growth of 1st crop (pot experiment)	0·3	0·3	0·6	0·2	1·0
Maximum growth of first four crops	0·3	0·3	—	1·0	1·0

The titration method therefore allows of a good approximation of the probable biological changes resulting from treatment of a soil with a given quantity of caustic lime. With respect to the crop producing power of treated soils the conditions are somewhat more complicated. Laboratory work has shown that the returns of nitrogen as ammonia and nitrate are fairly proportional to the amount of lime applied (i.e. within the first 9–12 months), but in heavily limed soils this nitrogen is largely in the form of ammonia. Consequently, plants growing in such soils obtain their nitrogen in a non-nitrate form and utilise it, as has been frequently demonstrated, in an uneconomical manner; hence no direct relation exists between the total ammonia and nitrate produced in two differently limed soils—in the one case where the nitrogen is chiefly in the form of nitrate, and in the other in the form of ammonia. The pot experiments carried out with the above soils have served to show, however, that an application to a soil of an amount of caustic lime equal to that indicated by the titration method results (*a*) in a maximum production of dry matter in the first crop - heavier applications tending to give a relative or actual depression, thus constituting "over-liming," and (*b*) in a maximum growth of the four crops used in our experiments. Hence the method proposed may serve as a safeguard against (1) "over-liming" and its undesirable consequences, and (2) the uneconomical utilisation of soil nitrogen.

When the application of lime is sufficient to impart an alkaline reaction to the soil water, certain other changes appear to be induced. During a number of titration tests with various soils, it was clearly evident that a flocculation of the soil compounds occurred when a slight excess of lime was present, and the time required for the passage of a definite amount of wash water (200 c.c.) approached a minimum as the partial sterilisation region was reached. This was so noticeable that some readings were taken, and these are plotted along with the titration curve and dry matter production in Curve 2. These data apply to Rothamsted soil, but similar results were also obtained with Millbrook soil. Thus a close agreement obtains between the results of nitrate determinations, tests for protozoa, pot culture experiments and the titration method suggested.



Curve 2. Showing the Effect of an Application of the Critical Dose of Calcium Oxide (as indicated by the Titration Method for determining the Partial Sterilisation Point) on Filtration and on Crop Production (Rothamsted Soil)

PART II. LIME REQUIREMENTS FOR NEUTRALISATION PURPOSES.

By far the most frequent purpose for which lime is employed in agricultural practice is the correction of "soil acidity," whether this is due to the presence of purely mineral soil constituents, to the application of acid artificial fertilisers, or to the accumulation of organic residues evidenced by more or less pronounced peat deposits. The condition is indicated in the field by the occurrence of certain "calcifugous" plants, e.g. *Rumex acetosella*, *Chrysanthemum segetum*, *Spergula arvensis*, and *Scleranthus annuus*, by the repeated failure of leguminous crops such as clover and lucerne, or by the incidence of "finger and toe" in cruciferous crops. Declining fertility of the soil gradually becomes evident, although this is subject to variation with the different crops—such crops as barley, wheat and clover being more, and oats, rye, millet, buckwheat and potatoes less sensitive, to soil acidity.

In the laboratory various tests have been employed for indicating the desirability of lime applications, and these may be divided into two classes according as to whether they indicated (*a*) the presence of a sufficiency of bases, usually calcium, or (*b*) lack of bases or soil acidity.

General Methods for indicating Lime Reserves in the Soil.

Of the various methods designed to indicate the presence of an adequate supply of base, those for the determination of free carbonates have been most generally adopted. These methods have, however, often differed greatly in procedure and the results do not admit of direct comparison in the majority of cases. Neglecting this fact, certain standard carbonate contents have been suggested, but the evidence in favour of these is very meagre, resting as it does chiefly on the observed fact that soils containing 0·5–1·0 per cent. calcium carbonate are generally fertile, other conditions being normal, although closer inquiry shows that many soils are devoid of carbonate and are still quite productive. The method has the advantage, however, of indicating the amount of lime present in a form capable of limiting soil acidity, whilst this can by no means be claimed for other methods where the amount of lime extractable by various solvents is calculated as carbonate. Hollemann (7) proposed extraction of the soil with water saturated with carbon dioxide and estimation of the lime removed; where the amount of lime thus extractable from a clay soil fell below 0·15 per cent. (CaO) a benefit might be expected from liming.

Immendorff (8) employed a method for indicating lime reserves in the soil, in which 2·5–10 grms. of the soil are boiled for half an hour with 200 c.c. of distilled water and 25 c.c. of sulphuric acid (N/5), the liquid being then filtered and a back-titration made. Where it is a question of ascertaining the amount of easily soluble alkaline earths irrespective as to whether these are present as carbonates or silicates the method is said to give useful results.

Sestini (9) suggests the use of boiling acetic acid and Mayer (10) also used 1 : 2 acetic acid on account of its inactivity towards ferrous carbonate. Extraction with hot dilute hydrochloric acid has been employed, and Heinrich (11) gives the following scale of minimum contents of lime (CaO) extractable with 10 per cent. acid. According to this a lime content of

0·05 % is sufficient for the growth of potatoes and rye,
0·05–0·10 is sufficient for the growth of oats and barley,
0·10 is sufficient for the growth of peas and vetches,
0·12 is sufficient for the growth of clover and
0·20–0·30 is sufficient for the growth of lucerne.

Maercker (12) gives two scales—one for sandy soils and another for loams—10 % hydrochloric acid being used, while Orth (13) gives still another for data obtained by the use of strong acid. It soon became evident that the lime thus removed from the soil could not fairly represent the amounts of lime available either for plant growth or for the correction of soil acidity, and other methods depending on the interaction of lime compounds in the soil with various neutral salts were suggested.

The one proposed by Stützer and Hartleb (14) rests upon the decomposition of soil carbonates by the addition of ammonium chloride, the ammonium carbonate formed being then estimated by distillation.

Following a method indicated by Kellner (15) for the determination of the absorptive power of soils, Meyer (16) elaborated one for the estimation of lime in an available state. This consists in the treatment of 10 grms. of soil with 100 c.c. of a 10 per cent. solution of ammonium chloride for three hours and the determination of the lime removed in the solution. Gregoire showed, however, that this period was not sufficient for the removal of the whole of the available lime and recommends extension of the period to 10 hours. In view of the fact that this method often gives anomalous results, Weibull (17) drew into consideration another factor—that of the organic matter of the soil as indicated by "loss on ignition." On the assumption that the lime (i.e. soluble in ammonium chloride solution) tends to react as an alkali, and the "loss on ignition" portion acts as an acid component there should obtain a definite ratio between these two for the production of a neutral soil reaction. According to Weibull, it may be stated that (1) ordinary soils with 3–6 % "loss on ignition" and less than 0·30 % soluble lime are acid in character and possess low nitrifying power; soils poor in lime and with a more neutral reaction have a lower "loss on ignition" content.

Soils with traces or more than traces of calcium carbonate, or soils without carbonate but with more than 0·25 % available lime, are, as a rule, alkaline and possess a marked nitrifying power; as exceptions are soils with high humus content. If the ratio lime: "loss on ignition" fell below 1 : 20 the soils examined were acid in character; if above 1 : 20 the reaction was alkaline. Meyer examined a number of soils in this respect and found good agreement on the whole between the lime content: loss on ignition ratio and the reaction of the soil.

TABLE III. *Relation between the Lime Requirements of some Danish Soils and the percentage of Lime soluble in Ammonium Chloride Solution (Christensen and Larsen).*

Percentage of lime (CaO)	No. of experiments	Results of field experiments Lime response			
		positive	negative	doubtful	none and doubtful
0- 0·05	16	87 %	6 %	6 %	13 %
0·06—0·10	16	87	6	6	13
0·11—0·15	17	65	24	12	35
0·16—0·20	15	40	40	20	60
0·21—0·25	17	24	65	12	76
0·26—0·30	8	0	88	13	100
over 0·30	27	11	81	8	89

His analytical data are, however, difficult of interpretation– one soil possessing 0·372 % soluble lime (CaO), 0·126 % CO_2 (= 0·286 % $CaCO_3$). an acidity of 0·054 % (expressed as CO_2) and an acid reaction, but did not respond in pot culture to an application of calcium carbonate. Of the six soils with which Meyer worked three possessed a lime content above the normal (0·25 %), five are stated to be acid in character, but only one was found to respond to carbonate applications. Briefly, the analytical data failed to throw any light on the need of lime. In connection with an extensive scheme of liming experiments initiated by the

Danish "Lime Committee," Christensen and Larsen correlated the results of the examination of a number of soils by this and other methods with the results of field trials. Those obtained with the ammonium chloride method are given below.

The probable "lime requirement" limit is thus about 0·16–0·20 %, but of the total number of cases there remain about 40 % in which little guidance is given by the method.

THE DETERMINATION OF SOIL "ACIDITY."

(*a*) *Qualitative Methods.*

The simple and commonly adopted test for soil acidity is that with litmus paper, in which a strip of neutral paper is interposed for upwards of 15 minutes between two masses of the moist soil to be tested. For rough work in the field the method possesses a certain amount of value – the production of a red tint generally being an indication of acid soil conditions. On the other hand, however, the failure to give any colour change does not necessarily mean that the soil would not respond to an application of lime. The Craibstone soil largely used in our experiments failed to react to this test, but responded distinctly to treatment with carbonate both in laboratory and pot culture experiments. A refinement of the method has been introduced by Christensen and Larsen (18), who used neutral litmus solution and classified the soils according to the tint produced in the test. In the test, 1 c.c. of a neutral litmus solution and 20 c.c. of distilled water are placed in test tubes of about 40 c.c. capacity; 5 grms. of soil are then added, shaken up, and allowed to stand until the following day. According to the tint produced the soils may be grouped into acid, weakly acid, neutral and alkaline types. Of the soils tested by these investigators, 26 were found to be acid or weakly acid in reaction and of these only one failed to respond to treatment in the field; of 50 soils found to give a neutral reaction, 58 per cent. still responded to treatment and 14 per cent. were doubtful, thus supporting the view expressed above with regard to this test.

Loew (19) employed a method based on the liberation of iodine from potassium iodide added to the soil and the production of a blue colour with starch, while Daikuhara (20) suggests the following test: 5 grms. of the soil are placed in a test tube and a 10 % solution of potassium nitrite (chemically pure, and especially free from carbonate) is added drop by drop until the soil is moderately moistened; the mouth of the tube is then closed with a plug of cotton wool from the middle of which a strip of potassium iodide starch paper is suspended. After a short period the degree of soil acidity can be gauged by the intensity of the blue colouration produced. Another method, suggested by Baumann and Gully (21), consists in the addition of a solution containing 2·0 per cent. potassium iodide and 0·1 per cent. potassium iodate to 1–3 grm. of the soil in a test tube; after being shaken and then allowed to stand for 15 minutes the solution is filtered and an equal quantity of dilute starch solution added. The intensity of the colouration provides an index of soil acidity.

In a test proposed by Albert (22) the soil is suspended in water, a few grains of lithium phosphate added and then allowed to stand until no further colour change occurs; the tint ranges from light brown to brownish-black and depends on the formation of soluble lithium humates.

(*b*) *Quantitative Methods.*

One of the earliest methods, and one which is still largely used on the Continent, is that introduced by Tacke (23) based on the capacity of the soil to liberate carbon dioxide from finely divided calcium carbonate. The soil is placed in a flask through which a slow stream of pure hydrogen is passed to remove residual carbon dioxide from the soil and the apparatus is then opened, by means of a T-piece, to a Pettenkofer absorption tube containing N/5 or N/10 sodium hydroxide solution; an excess amount of a suspension of finely divided calcium carbonate is then allowed to flow from a separating funnel to the soil bottle. The passage of hydrogen is then continued for 2½ hours and the amount of carbon dioxide evolved is estimated by titration of the soda against phenolphthalein after the addition of barium chloride solution. The method has given satisfactory results on the whole and has the advantage over various other methods in that the change upon which it depends is the one occurring naturally in the field; its disadvantages are that the determination is somewhat wearisome and that, during the period of the determination, there proceeds a slow but appreciable degradation of organic matter with the production of carbon dioxide. To obviate this source of error, Süchting (24) has suggested the employment of a definite amount of calcium carbonate and the estimation of the residual carbonate after all change has ceased.

Wheeler, Hartwell and Sargent (25) attempted to render the Tacke method more rapid by boiling the mixture, but found that degradation changes proceeded still more rapidly and resulted in fictitious values being obtained.

The capacity of the soil to absorb the base from neutral salts in solution has formed the basis of a number of methods. In their earlier work, Hopkins, Knox and Pettit (26) recommended that 100 grms. of dry soil be digested in the cold for three hours with 250 c.c. of a 5·0 per cent. solution of sodium chloride, the whole being shaken at intervals. At the end of this period 125 c.c. of the clear supernatant liquid was syphoned off and, after being boiled for a few minutes to drive off carbon dioxide, was titrated with standard alkali against phenolphthalein. Actual experiment showed that displacement proceeded until an equilibrium was established and the removal of the liquid and successive treatment of the same soil with fresh quantities of solution resulted in values being obtained which were in the order of decreasing geometrical progression. For routine work, however, it was found convenient to employ a factor—the results of the titration of the first 125 c.c. 3 giving results equal to those obtained by successive digestions. The factor subsequently suggested was 4, while still later (27), where a normal solution of potassium nitrate was employed, one of 2½ was advised. The earlier method has been criticised by Daikuhara (28), who was able to show that the values given by sodium chloride were very much lower than those obtained when other -chiefly ammonium and potassium salts were used, and suggests that potassium chloride be employed. Daikuhara's own work shows also that this salt gives values very similar to those obtained with potassium nitrate, and he does not appear to be aware that the latter salt had already been recommended by Hopkins. The factors given by Daikuhara are 3·5 and 3·0 for digestions of one and five days respectively. Employing this method, he examined a very large number of so-called "acid mineral soils," such as appear to be widely distributed throughout Japan and Korea, and the results present several points of interest. One characteristic of the soils is that the application of neutral mineral manures results in decreased crop yields, whilst the zinc pots in which the experiments were made were gradually attacked and finally perforated. The soils fail to give an acid extract with water and give a reaction with litmus paper only at the point of contact with the soil. With neutral salt solutions an acid reaction is produced immediately and this is advanced as a test for such acid soils. It is important to note, however, that of 917 Japanese virgin soils, 738 gave a response to litmus paper and only 467 reacted to this test; the method is not, therefore, generally applicable.

The detailed analysis of the potassium chloride solution after digestion with the soil had taken place showed that an interchange of bases had occurred and large quantities of alumina and iron had come into solution in the form of acid salts—in fact, the characteristic reaction of the soils is attributed to the presence of alumina[1] and iron compounds which are loosely absorbed by soil colloid bodies.

Two other methods, in which salts of weaker acids are used, have been described. According to Loew (29) 50 grms. of finely ground air-dry soil are digested with 200 c.c. of neutral 1·0 per cent. solution of sodium or potassium acetate at room temperature for 24 hours. The acetic acid is more easily displaced than the stronger mineral acids and is estimated by the titration of an aliquot portion of the filtrate.

The bases employed by Loew are obviously not such as would come into consideration for the correction of soil reaction in the field, and Jones (30) has accordingly used the calcium salt; 5·6 grms. of soil are ground in a mortar with 0·5 grm. neutral calcium acetate and then sufficient water is added to make a stiff paste. Grinding is continued and a further 30 c.c. of water added and mixed for 30 seconds; the whole is then transferred to a 200 c.c. measuring flask, the volume made up to about 160 c.c. and allowed to stand (with occasional shaking) for 15 minutes, after which water is added to make up to 200 c.c. and filtered. The first 10 or 15 c.c. of the filtrate are rejected and a further 100 c.c. taken for titration with phenolphthalein. This reading × 2 × 1·8 × 1000 gives the amount of lime[2] in lbs. per acre of two million pounds of soil. The method is extremely rapid, but in our own tests has given far from satisfactory results.

A further group of methods rests in the absorptive capacity of the soil for various alkaline compounds. In that of Wheeler, Hartwell and Sargent (31) the soil is digested at room temperature for 42 hours with a known volume of approximately N/10 ammonia. A portion of the clear supernatant liquid is withdrawn and hydrochloric acid added to throw down the humic acid; after filtration the excess of acid is estimated by titration. A colorimetric method was also attempted by these authors. These and similar methods involving the use of alkaline compounds are, however, open to the objection that whether the soil be acid or neutral a considerable amount of the compound is directly adsorbed and we have no means of determining this amount. The experiments in the first part of this paper will suffice to demonstrate this adsorption - such neutral soils as the Rothamsted and Chelsea samples exhibit marked powers for taking up calcium hydroxide. Furthermore, where ammonia is used it is practically impossible to obtain a clear filtrate or supernatant liquid. As an alternative to Tacke's method, Albert (32) proposed one in which the absorptive capacity of the soil for calcium, magnesium or barium was determined, the latter being preferred on account of the lower dissociative power of its salts. For the determination, 20–50 grms. of the air-dry soil (according to acidity) are placed in a Jena glass flask together with 200 c.c. of distilled water, 50–100 c.c. of N/5 barium hydroxide solution are run in from a burette and 10 grms. of solid ammonium chloride added. The flask is fitted immediately to a condenser and the contents boiled for 20–25 minutes, the ammonia evolved being collected in N/10 acid and a titration made with sodium alizarin sulphonate as indicator. Since the amount of the ammonia displaced from the ammonium chloride is equivalent to the amount of barium hydroxide not absorbed by the soil, this absorption or the soil acidity may be determined. The values obtained by the use of barium, calcium or magnesium hydroxide were found to vary widely among themselves. According to Suchting and Arnd (33), Albert's method is of little value since the results are distinctly dependent on the period and intensity of boiling.

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[1] The unproductiveness of certain American soils has been ascribed to the presence, in the soil, of soluble aluminium salts (Abbott, Connor and Smalley, Purdue Univ. Agric. Exp. Stat., *Bull.* 170. 1913).

[2] Chalk ($CaCO_3$) is evidently intended, and not lime (CaO) as stated in the original paper.

Lyon and Bizzell (34) have recently introduced a modification of Albert's method. The reaction between soil and alkali does not occur immediately and these workers recommend the addition of the barium hydroxide solution about 60 minutes before that of the ammonium chloride—the soil and alkali being kept at boiling point in the meantime. The procedure allows of two sources of error; the first is due to the capacity of all soils to expel a quantity of ammonia from the ammonium chloride employed when the mixture is subjected to heat, and Lyon and Bizzell suggest a correction for this - a blank determination being made with soil and ammonium salt but without alkali. In some of our own work with this method the amount of ammonia thus expelled by a soil deficient in lime and with the addition of the prescribed quantity of ammonium chloride amounted to 20 25 per cent. of the total produced on the addition of barium hydroxide as in the standard method; the distillation of a further 50 c.c. increased this error to over 30 per cent. In the second place, all soils liberate appreciable quantities of ammonia when heated with barium hydroxide solution at 100° and the amount thus formed must be taken into consideration.

A method which has, perhaps, been more extensively used in the United States than any other is that introduced by Veitch (35). In this, three lots of soil each of 10 grms. are weighed into platinum basins and about 50 c.c. of water added; three different volumes, say 10, 20, and 30 c.c. of standard calcium hydroxide solution are added and the basins are placed immediately on a water bath and their contents evaporated to dryness. With the aid of 100 c.c. of distilled water the soils are then transferred to Jena glass flasks and allowed to stand overnight: the reaction of about 50 c.c. of the filtered liquid is tested by boiling with phenolphthalein, the volume being reduced by boiling, if necessary, to 5 c.c. The three quantities of lime-water taken will generally suffice for orientation—one of the quantities being too small and the others too large for exact neutralisation or *vice versâ.* Within the limits thus set by the trial determination, other tests are made where the amounts of lime-water vary by no more than 2 c.c.; the approximate requirement may thus be obtained.

The method is based on the fact that the slight excess of lime-water present after the neutralisation of the acid soil material is converted into carbonates or bicarbonates, the boiling solution of which gives an alkaline reaction with phenolphthalein. The strict adherence to the prescribed directions in these methods is essential the adoption of an extended period of digestion as, for instance, in the Albert method, or failure to place the soils at once on the steam bath as in Veitch's method would probably lead to untrustworthy results.

Wheeler and his colleagues and also Veitch attempted to obtain a measure of soil acidity by digestion of the soil with lime-water in the cold. The results thus obtained by Veitch were higher than those given by his acidity method, and there appears to be no reason to doubt that the introduction of the heat factor is responsible for this difference.

Gregoire, Hendrick, Carpiaux and Germain (36) have recently investigated the action of soils on a "Kjeldahl's solution" of the following composition:

55·3	grms.	potassium iodide,
14·3	,,	potassium iodate,
99·2	,,	sodium thiosulphate (cryst.) and
1000	c.c.	water.

For titration against this solution a second one containing 17·0 grms. iodine and 25·0 grms. potassium iodide per litre was used. The strength of the iodide solution is determined by taking 15 c.c. of the "Kjeldahl's solution" together with 20 c.c. of N/5 hydrochloric or sulphuric acid and titrating the excess of thiosulphate with the iodine solution, the difference between the value thus found and that of a direct titration of the "Kjeldahl's solution" being the one required.

For the determination of acidity, 10 grms. of the fine soil (passing through a 1 mm. sieve) are digested in a flask with 15 c.c. of the "Kjeldahl's solution" for a prescribed period, after which the volume is made up to 110 c.c. and 100 c.c. taken for titration. The results are chiefly expressed as H ions per kilo of soil. The amount of reaction with the soil depends, in the first place, on the concentration of the solution, and the above strength is recommended; it has further been found that the change is not immediate but continues for a considerable period with all soils, although where the soil is acid the greatest amount of change takes place within the first 24 hours. The slight additional reaction between this time and the end of 15 days is common to all soils and is doubtless due to interaction with inorganic soil constituents. It must be noted that a positive reaction is given even when the soil contains large amounts of carbonate and the setting of a limit of maximum absorption for neutral soils appears desirable.

A point brought out by the results of Gregoire and his co-workers is that a fairly large proportion of soils occur that not only do not possess any lime as carbonate but also fail to give any definite reaction as to acidity; they are, in fact, just neutral and an estimation of carbonate alone would fail to give reliable information as to the lime requirements of such soils.

Experimental.

In the course of our other work on the relative action of calcium oxide and carbonate on the soil, the need was felt of a simple and accurate method for the determination of the lime requirements of these soils. In view of the fact that carbonates and bicarbonates are the chief compounds tending to maintain a neutral reaction in the field, and that any amelioration must proceed through this change, it appeared likely that a closer investigation of the action of certain carbonates on the soil might give a measure of prevailing acidity, and would possibly conform more closely to natural conditions than some of the methods hitherto employed. Preliminary work with sodium carbonate and bicarbonate gave unsatisfactory results inasmuch as deflocculation of the clay compounds of the soil occurred and adversely affected the rate of filtration: a coloured extract difficult of titration was obtained and, furthermore, positive absorption was shown even in the case of neutral soils containing an abundance of carbonate initially. Some of these results[1] are given below.

TABLE IV. *The Absorptive Power of Soils for Sodium Carbonate.*

Soil	$CaCO_3$ content of soil	Compound employed	Absorption (stated as $CaCO_3$ required)
Rothamsted	2·6 %	Sod. carbonate	0·390 %
"	"	" bicarbonate	0·125
Oundle	over 40 %	" "	0·175
Woburn	nil	" "	0·175
Craibstone	nil	" "	0·290

In view of these unsatisfactory results recourse was then had to the use of a solution of calcium bicarbonate, and after minor modifications as to period of digestion, strength of solution, etc., this method has been used with a large number of soils under well controlled conditions.

The Method. The required solution may be prepared either by passing a current of carbon dioxide into a suspension of calcium carbonate in distilled water, or by means of a "Sparklet" or refillable soda-water syphon, for which bulbs of compressed carbon dioxide are used. The latter method is the more convenient and permits of the preparation of a saturated solution within quite a short time. A large excess of carbonate must be used (about 10 grms.) in order to provide an abundance of small particles which pass readily into solution and the syphon requires occasional gentle shaking for about 15-20 minutes. The contents may then be diluted with one-third its volume of distilled water and filtered; this will result in the formation of a solution of approximately N/50 strength.

For a determination of acidity, or lime requirement, 10-20 grms. of the soil are placed in a bottle of 500–1000 c.c. capacity together with

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[1] For general convenience and purposes of comparison the data from our soil examinations are stated in terms of calcium carbonate required to bring the soil to the neutral point. This allows of direct interpretation for general field work, each 0·1 per cent. being taken as equal to 1 ton per acre of 2½ million pounds of soil. In specific cases it is advisable that the apparent specific gravity of the soil be determined.

200-300 c.c. of approximately N/50 solution of calcium bicarbonate, and the air in the bottle is displaced by a current of carbon dioxide in order to insure against possible precipitation of the calcium carbonate during the period of the determination. The bottle is then placed in a shaking machine for three hours[1], after which time it is opened, the liquid is filtered, and a portion of the filtrate equal to half the original amount of bicarbonate solution is titrated with N/10 acid using methyl orange as indicator. The difference between this final titration and that of the initial solution represents the amount of calcium carbonate absorbed, each cubic centimetre of N/10 acid being equal to 5 mgrms. of calcium carbonate.

Preliminary work with this method served to show that with neutral soils there was practically no absorption, while the presence of carbonate in a soil often resulted in an increase in the strength of the solution during the period of the determination. For all our routine work a small rotary shaker was used which gave a gentle agitation to the contents of the bottles.

The extent of interaction between soil and solution obviously depends largely on the rate at which the soil particles are broken down, and in order to ascertain the minimum time required for this action a very unkind acid Oxford clay soil was used after being passed through a 3 mm. sieve. The results are included in the following table.

Soil	Per cent. absorption after						
	1 hour	2 hours	3 hours	4 hours	6 hours	9 hours	11 hours
Ridgmont	0·38	0·45	0·40	0·43	0·43	0·43	0·44

In view of the relatively high absorption after two hours and the lack of increase after this period, even with this heavy soil, we felt justified in taking three hours as the maximum period for further work.

As might be anticipated the strength of the bicarbonate solution regulates the amount of absorption with any given soil and it appears important that, for the reaction to approach completion within the prescribed period, the concentration of the initial solution should not be much below N/50 strength. The results of a comparative experiment will suffice to illustrate this.

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[1] Occasional shaking by hand (at intervals of 20 minutes) for four hours, gives identical results.

Initial concentration of solution	N/50	N/75	N/100
Absorption (as per cent. of soil)	0·272	0·265	0·210

Adhering to this period of digestion and N/50 strength of solution the method has been tested against some of those reviewed above, our routine soils being employed. The results of some of these comparisons are given in Table V.

TABLE V. *Comparison of various Methods for determining Soil Acidity.*

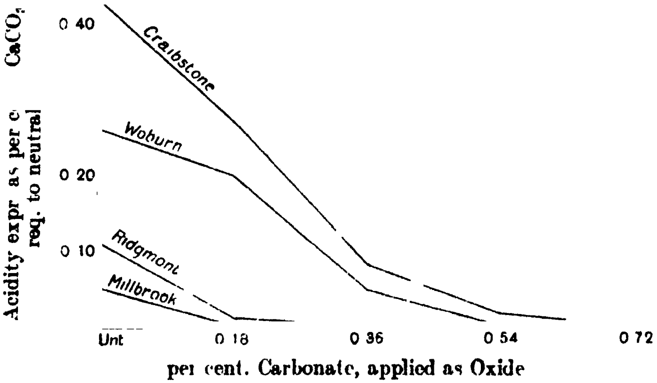
Method used after	Acidity expressed as $CaCO_3$ required to neutralise the soil (per cent.)				
	Chelsea	Millbrook	Oundle	Woburn	Craibstone
Jones	0·045	0·045	0·018	0·232	0·161
Hopkins	0·012	0·006	0·002	0·244	0·030
Lyon and Bizzell	-	—	—	0·226	0·436
Veitch	—	--	-	0·204	0·407
Bicarbonate method	nil	0·020	nil	0·260	0·430

Where the soil reaction is presumably due to the presence of acid mineral compounds, as in the case of the Woburn soil, the methods proposed by Jones and Hopkins give results closely approaching those obtained by other methods, but this agreement no longer holds when the reaction or lime requirement is due to other causes, as in the Craibstone soil. It is interesting to note that in both cases the bicarbonate method gives data agreeing well with those from the more laborious Lyon and Bizzell and the Veitch tests.

In connection with routine bacteriological and chemical work, certain of the above soils had received various additions of calcium oxide and carbonate and served as control material for testing the accuracy of the method. Although the response of a soil to the correction of acid conditions can only be determined generally by carbonate applications, on account of the possible direct chemical action of the oxide on some soils, evidence will be adduced later which tends to show that so long as the neutral point is not reached, the value of oxide for neutralisation purposes is practically the same as that of carbonate. We feel justified therefore in using some of these soils to show the reduction of acidity as indicated by the bicarbonate method. Some of the data from such determinations are given in Table VI, and plotted in Curve 3.

TABLE VI. *The Acidity of certain Soils after Treatment with Lime.*

CaO applied (stated as $CaCO_3$ per cent.)	Rothamsted	Millbrook	Woburn	Craibstone	Ridgmont
0	0	0·05	0·26	0·43	0·11
0·18	0	0	0·20	0·27	0·02
0·36	0	0	0·05	0·08	0
0·54	0	0	0	0·02	0
0·72	0	0	0	0	0



Curve 3. The Reduction of Acidity in Soils treated with Calcium Oxide and stored in a moist condition.

The reduction in acidity with increasing applications of lime is, in the case of the Woburn soil, gradual and not strictly proportional to the amounts applied, but this is not surprising in view of the fact that the soil during the period of storage only contained initially about 18 per cent. water and that some of the lime applied could not be expected, on account of the relatively large size of the particles, to come into action with rapidity. As against this we have the case of the Craibstone soil where the reduction in acidity is directly equivalent to the amount of lime supplied in the two lower dressings. Beyond this there occurs a perceptible "lag" in the curve.

The relation between soil acidity and responsiveness to the application of calcium carbonate is well demonstrated by the increased rate of ammonia and nitrate production (as an indication of increased bacterial activity) and the amount of plant growth in these soils.

Full details have already been given elsewhere (37), but the following table will serve to bring out this relation more clearly.

TABLE VII. *The Relation between Soil Acidity, the Production of Ammonia and Nitrates, and Plant Growth in certain Soils.*

Soil	Lime req. (as per cent. $CaCO_3$)	Increase in ammonia and nitrates (parts per mill. dry soil)		Plant growth (production of dry matter in 4 crops)	
		Unt. soil	Soil + $CaCO_3$	Unt. soil	Soil + $CaCO_3$
Rothamsted	nil	7	6	100	105
Chelsea	nil	49	44	100	99
Millbrook	0·02	7	12	100	97
Woburn*	0·26	40	84	100†	740†
Craibstone	0·43	26	47	100	144

* The authors desire to express their indebtedness to Dr J. A. Voelcker, Director of the Station, who very kindly placed this and other samples of soil at their disposal.

† From other experiments, first crop only.

The two soils, Rothamsted and Chelsea, contained initially an abundance of carbonate (2·6 and 0·8 per cent. respectively) and the further addition of chalk gives returns varying only slightly from those of the control soils; Millbrook soil, which is almost neutral, gives a slight increase in nitrates and a depression in plant growth. The two acid soils, Woburn and Craibstone, respond readily to chalk applications as indicated by increased nitrate production and plant growth in the first four crops after treatment.

In another experiment the addition of calcium carbonate to a soil showing a lime requirement of 0·117 per cent. resulted in an increase of the following barley crop of 30 per cent.

Having shown that the method suggested provides a good indication of preceding treatment of the soil in the laboratory, some further determinations with field soils may be of interest. The samples used in these experiments were obtained from the permanent wheat and barley plots of the Woburn Experimental Station, already well known on account of their pronounced acid character as a result of continued applications of sulphate of ammonia. Up to 1897 the crops on some of these plots had already begun to fail, while a preliminary application of lime sufficed to bring crop production to its normal level. Since 1898 various other dressings with lime have been made, in each case with marked benefit (38), and the effect of these dressings is reflected in the reaction of the samples at the present date. For purposes of comparison we have included in the following table the carbonate content of the soils and the yields of barley for the 1913 crop. Full details of the manuring, etc., are to be found in the official reports of the Woburn Station.

TABLE VIII. *The Lime Requirements of Certain Field Soils.*

(Permanent Barley Plots, Woburn Experimental Station.)

Soil treatment	Lime (CaO) applied equiv. to $CaCO_3$ per acre	$CaCO_3$ present 1914	$CaCO_3$ required 1914	Crop yield 1913 bushels
2a sulphate of ammonia (25 lb ammonia)	—	0·003 %	0·260 %	—
2aa as *2a*, with 5 cwt lime 1905, 1909, 1910 and 1912	1·8	0·008	0·140	11·9
2b as *2a*, with 2 tons lime 1897, reptd. 1912	7·2	0·196	—	34·3
2bb as *2b*, with 2 tons lime reptd. 1905	7·2	0·026	0·043	16·3
5a mineral manures and sulph. ammonia	–	–	0·183	—
5b as *5a*, with 2 tons lime 1897 reptd. 1912	7·2	0·111	—	31·5
5aa as *5a*, with 1 ton lime 1905	1·8	0·006	0·140	13·1
8a and *8b*, mineral manures and (in alternate years) sulphate of ammonia (50 lb ammonia)			0·190	-
8aa and *8bb* as *8a* and *8b*, with 2 tons lime, 1897 reptd 1912	7·2	0·111	–	31·6
1 and 7 unmanured	—	0·006	0·123	6·7

Without necessarily indicating that the controlling factor in crop production of these plots is one of physiological resistance to soil acidity, there is still a very close agreement between yields and soil reaction. In all cases where the soil is neutral in reaction, high returns are obtained; where the requirement is more than 0·18 per cent. the crop shows almost if not complete failure. In addition to an improvement in conditions for plant growth, bacterial processes are speeded up so that, while in our experiments the amount of ammonia and nitrate formed in the untreated soil within the first fifty days was only 7 parts per million, that in soil with only 0·2 per cent. calcium oxide reached

26 parts. Somewhat similar data were obtained with the soils from the permanent wheat plots, although in this case the crop was more resistant to acid conditions, and persisted until the soil showed an absorption of over 0·22 per cent.

The Relation between Lime Requirements and Calcium Carbonate Content.

The relation between the lime requirements of a soil and the amount of calcium carbonate contained therein is of importance in certain circumstances. In the first place, the mass of data now being collected under numerous soil survey schemes is open to misinterpretation on account of the view commonly held that normal soils must contain a certain (often purely arbitrary) content of carbonate. Secondly, and related to the first consideration, field experiments which are initiated on the basis of a low carbonate content of the soil are sometimes liable to give only negative results. This has already occurred from time to time.

Hall and Russell (39) and later Gregoire and his colleagues record a considerable number of soils neutral in character and yet possessing little or no carbonate. We have also met with many of this type, chiefly in our case, light sandy soils. Conversely, many acid soils are encountered where a certain amount of carbonate is present, but this appears to be largely due to localisation of the carbonate (posssibly past applications of lime) in the field. Some examples of each type may be found in the following table, and the authors wish to emphasize the fact that acidity and not merely carbonate determinations should be made in estimating the needs of any particular soil.

	Rothamsted	Chelsea	Devon	Millbrook	Geescroft	Metchley	Harper Adams	Woburn	Craibstone	Leeds II
$CaCO_3$ present %	2·660	0·890	0·003	0·035	0·005	0·097	0·005	0·003	nil	nil
$CaCO_3$ req. %	nil	nil	0·015	0·032	0·100	0·117	0·135	0·260	0·430	0·470

The Relative Values of Calcium Oxide and Carbonate for Soil Neutralisation.

Attention has already been called to the essential difference in action between oxide and carbonate when added to neutral soils, the former exercising a specific effect. In the case of soils lacking in lime this difference was not distinctly evident, but on account of having in most cases applied a large excess of carbonate it was difficult to draw fair comparisons with these soils. It was evident however that in the case of the Woburn and Craibstone soils, the net effect of the two forms of lime was approximately the same and it was decided to carry out pot experiments in order to bring out this similarity if possible.

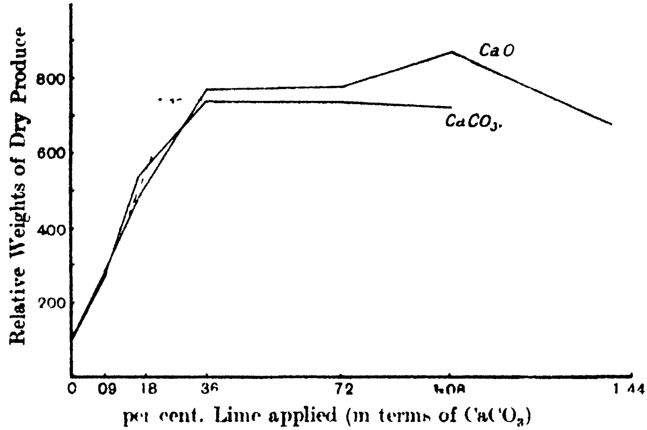
Small glazed earthenware pots were filled with equal quantities (3 kilos) of Woburn acid soil and various dressings of calcium oxide and carbonate (in equivalent quantities) were made, in each case in duplicate. The soils were watered after treatment and in order that crop growth should not be limited by lack of potash and phosphates, an addition of 0·5 grm. of potassium phosphate (neutralised in solution to litmus) was made to each pot of soil. After being allowed to stand in a moist condition for about seven weeks, the soils were stirred up, barley seed was sown and the plants allowed to grow to maturity, that is, about fourteen weeks from the date of sowing. From germination onwards little difference was perceptible between the effect of the two forms of lime and this was also borne out by the actual weights of the plants when cut. These dry weights and relative weights are included in Table IX and are also plotted in Curve 4.

TABLE IX. *The Relative Values of Oxide and Carbonate when applied to Woburn acid soil.*

Crop, Barley.

	Oxide		Carbonate	
	Dry weight	Relative	Dry weight	Relative
Soil untreated	1·52 grm	100	1·52 grm.	100
Soil + 0·05 % CaO or 0·09 % $CaCO_3$	4·25	279	4·08	268
Soil + 0·10 % „ or 0·18 % „	7·25	477	8·27	544
Soil + 0·20 % „ or 0·36 % „	11·67	768	11·27	741
Soil + 0·40 % „ or 0·72 % „	11·78	775	11·21	737
Soil + 0·60 % „ or 1·08 % „	13·17	866	10·69	703
Soil + 0·80 % „	10·26	675	—	—

These results possess several points of interest. In the first place the returns in plant growth are almost directly proportional to the lighter dressings of carbonate supplied. Little difference is evident between the yields resulting from the application of the lower equivalent doses of oxide and carbonate until the requirements of the soil are satisfied, i.e. the neutral point[1] -the slightly lower value with 0·10 per cent. of oxide being due to an exceptional variation in the duplicates, the other value being 8·20 grms. as against 8·27 grms. with carbonate.



Curve 4. The Relative Effect of Calcium Oxide and Carbonate on Plant Growth in an acid Soil (Woburn) Crop, Barley

Secondly, the specific action of the oxide only becomes evident with 0·6 per cent. while the next higher dressing with this compound produces a relative depression. These two high dressings were, in fact, made on the results of the titration method described in the first part of this paper, where it is shown that 0·6 per cent. constitutes the critical dressing capable of producing the maximum first crop, while 0·8 per cent. is too high for this soil.

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[1] This is of considerable practical importance; the view is often expressed, and great stress has been laid upon it in the United States, that carbonate of lime (chiefly ground limestone) is the only form of lime that can be safely used in practice, on account of the tendency of caustic lime to "burn up" the soil. The results obtained by us in laboratory and pot culture experiments appear to denote that until the reaction of the soil is fully corrected, various decomposition changes proceed with similar rapidity with either form of lime It is only when excess quantities of oxide are employed—and this appears to have been the case in several American and English experiments—that a sudden liberation of soluble nitrogen compounds occurs in the soil, and local circumstances then determine whether this plant food is assimilated by the first crop or is subject to leaching during the following rainy season. If the latter takes place a heavily limed plot is liable to become impoverished to a greater extent than less heavily or even unlimed soils, and by decreased fertility in subsequent years tends to give support to the prevalent view of the "burning" action of the caustic form.

Finally, the results are of interest in connection with the question of acidity as indicated by the suggested bicarbonate method. Although maximum growth of the crop is produced by an application of 0·36 per cent., the preceding deviation in the curve might fairly be taken as an indication that this amount is actually in excess of the requirements. If therefore the two linear portions of the curve—the initial and the final be continued as is shown by the dotted lines, it will be found that the point of intersection occurs above the point already indicated as being the acidity by the bicarbonate method, namely, equal to 0·260 per cent. calcium carbonate. Without wishing to imply that with all soils the crop growth is directly in inverse ratio to the acidity of the soil, a close agreement appears to occur in this case between the lime requirement as shown by the bicarbonate method and the physiological gauge set by the plant.

The Relation between Soil Reaction and Bacterial Activity.

In discussing the necessity of maintaining an adequate supply of base in cultivated soils, it is generally recognised that the presence of carbonate is requisite for the maximum activity of nitrifying and nitrogen-fixing organisms, but little attention is paid to the preceding putrefactive or ammonification process. The work of Russell (10) and others shows that the amount of nitrate formed in field soils is strictly dependent on this preceding change, in fact, the ammonia formed is nitrified almost as soon as produced, hence the ammonia content of normal soils is invariably low.

In our own work with the Craibstone soil (which is free from carbonate) the amount of free ammonia is kept at a low level by continuous nitrification, while even with the Woburn soil the reserves derived from the fertilisers applied are subject to a steady change into nitrates. It appeared of interest, therefore, to ascertain how far a supply of calcium carbonate would affect each of these processes in the Craibstone soil, and two experiments with this end in view were made. It was obviously necessary for the demonstration of increased ammonification that nitrification should be eliminated and this we accomplished by treating the soils with toluene. Equal lots of 600 grms. of the moist soil were filled into bottles and these were divided into three sets. Of these the soil in set (*a*) was allowed to remain untreated, that in set (*b*) was treated with 10 c.c. of toluene per bottle, while that in set (*c*) received the same quantity of toluene and 2·0 per cent of calcium carbonate. After two days the toluened soils were spread out on sterilised paper in order to allow the antiseptic to evaporate and then returned to the bottles. The analytical data are given in Table X.

TABLE X. *The Effect of Calcium Carbonate on Ammonification.*

Treatment	Ammonia and nitrate (expressed as pts. N per million of dry soil) produced after					
	32 days			70 days		
	Ammonia	Nitrate	Total	Ammonia	Nitrate	Total
Untreated soil	2	39	41	—	44	44
Soil + toluene	17	35	52	27	31	58
Soil + toluene + $CaCO_3$	26	29	55	47	30	77

With the cessation of nitrate production the effect of calcium carbonate on ammonia formation becomes evident after 32 days, but this becomes still more pronounced by the end of the second period of the experiment, the gains during this time with the untreated soil, toluened soil and toluened soil with carbonate being 3, 6, and 22 parts of nitrogen respectively. It is evident therefore that ammonification is subject to retardation in this soil.

TABLE XI. *The Effect of Calcium Carbonate on Nitrification*

Treatment	Nitrate (expressed as pts. N per million of soil) produced after	
	20 days	57 days
Untreated soil	43	44
Soil + 0·10 % $(NH_4)_2SO_4$	77	131
Soil + 0·10 % $(NH_4)_2SO_4$ + $CaCO_3$	199	205

The second experiment consisted in the determination of the nitrates produced on the addition of ammonium sulphate to the soil, the various sets comprising (*a*) soil alone, (*b*) soil with 0·10 per cent. ammonium salt, and (*c*) soil with a similar amount of ammonium salt and 2·0 per cent.

calcium carbonate. The results, contained in Table XI, show the very great rapidity with which this change can proceed in the presence of carbonate, the change having reached completion within the first 20 days. The amount of nitrification taking place in the soil without lime was also quite appreciable in spite of the lack of free carbonate, although calcium may be liberated from the mineral or the organic soil constituents.

The main effect of an application of carbonate to this soil consists apparently in speeding-up both of ammonification and nitrification, but on account of the reasons stated above, the former action is probably the more important.

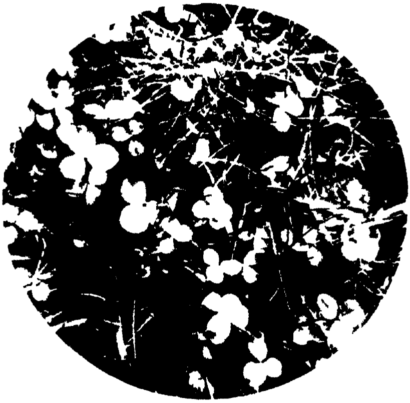
The Relation between Soil Acidity and Natural Vegetation.

While the conventional method of soil analysis with reference to lime generally consists in an estimation of carbonates, there are many instances in which this procedure fails to give an index to differences often apparent in the field. This applies to the distribution of plants on many of the heaths and commons throughout the country and also to a certain extent to the vegetation prevailing on much old grass land. As an instance, the case of the Harpenden Common may be cited, where carbonate determinations in soil samples from parts of the Common have failed to provide data that would help to grade the soils as to their relations to plant growth. All these soils react acid to litmus, but examination shows that from place to place, vegetations occur in which white clover, fescues, gorse, sorrel, bracken, etc., predominate.

A large number of these soils have now been examined by means of the bicarbonate method described above and we have been enabled to classify them in terms of acidity and vegetation. A few of these results are given below to illustrate some of the differences encountered. Photographs of typical turfs are shown in Plate I.

TABLE XII. *Lime Requirement as related to Vegetation.*

Average lime requirement of soil	Dominant flora
Approx. 0·22 % $CaCO_3$	Wild white clover (*Trifolium repens*)
„ 0·26 % „	Fescues (*F. ovina* and *rubra*)
„ 0·31 % „	Mixed. Yarrow, woodrush and moss
„ 0·39 % „	Gorse
„ 0·43 % „	Yorkshire fog
„ 0·53 „	Sorrel



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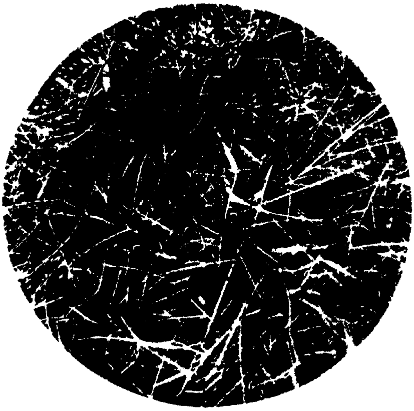

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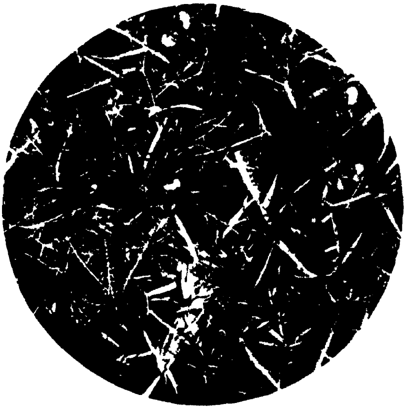

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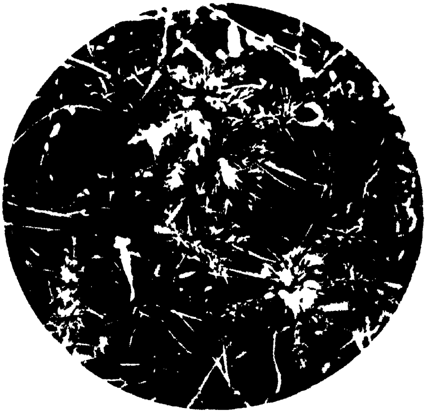

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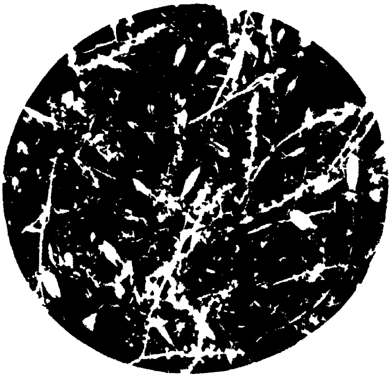

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Lime Requirement in Relation to Natural Flora. Photographs of turfs from Harpenden Common. The various soils showed lime requirements of (1) 0·200, (2) 0·255 (3) 0·282 (4) 0·353 and (5) 0·493

This gradation appears to apply quite generally to the soils on this formation (clay with flints, overlying chalk), but need not necessarily mean that on other and different soils the above plants are associated with the acidities[1] already given. There seems to be little doubt from general observation that clovers are least and sorrel most acid-resistant (neglecting the terms calciphagous and calcifugous), but the extent to which these various plants persist on any given soil is probably determined partly by reaction and the amount of water contained by that soil at any period. In this connection organic matter by helping to retain soil water might conceivably play a part in regulating these inter-relations, which go to form a subject of agricultural importance and of ecological interest.

SUMMARY.

The experimental results described in the two parts of the paper may be summarised thus:

PART I. *Lime Requirements for Sterilisation Purposes.*

(1) The capacity to produce partial sterilisation effects is a property belonging to calcium oxide (caustic lime), but not to calcium carbonate (chalk, limestone, marl, etc.).

(2) The amount of lime necessary to produce specific effects in different soils has been found to vary greatly and it is not possible to make any general recommendations.

(3) The method proposed for indicating the critical amount required is based on the determination of the amount necessary for the production of an alkaline reaction of the soil water.

(4) The amounts thus indicated agree very closely with those required for the production of typical partial sterilisation effects in the soil itself, e.g., the inhibition of protozoa and of nitrifying organisms.

(5) By correlation of the results obtained by the proposed method with those of pot experiments, it is evident that the amount indicated coincides with that required for (*a*) the maximum production of dry matter in the first crop following treatment heavier applications tending to be injurious, and (*b*) the maximum production of dry matter in the first *four* crops. Applications of lime double or treble the amount indicated by the method, although causing an increase in the

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[1] The types of "soil acidity" or lime requirement vary greatly and require further study in their relation to plant growth.

NOTES ON SOME METHODS FOR THE EXAMINATION OF SOIL PROTOZOA.

By C. H. MARTIN, M.A., and K. R. LEWIN, B.A.

(*Rothamsted Experimental Station.*)

(With Plates II and III.)

I. Introduction.

During the last three or four years, the protozoa of the soil have been the object of a considerable degree of interest, and investigations into their occurrence and importance have been made by workers here and elsewhere. The aim of the present paper is to indicate what we know of the life of the protozoa in the soil, and to furnish descriptions of certain methods which have been found useful in work on this subject.

When attention was directed to the protozoan inhabitants of soils, it was quickly found that protozoa in great numbers and variety were easily obtained by inoculating soil into a suitable medium. Setting out from this fact, investigators have frequently been led to describe the forms found in cultures from a soil as the fauna of the soil, thus making the more or less tacit assumption that every form found in cultures from a soil was leading a trophic life in the soil at the moment when the culture was made.

Unfortunately, what may be termed the "cultural fauna" of a soil is of relatively little value in forming an idea of the protozoa actually living in the soil. On the one hand the cultural fauna consists in part of protozoa which were present in the soil only as cysts; on the other, some forms relatively important in the soil, notably the thecamoebae, appear very late, or not at all, in cultures on the ordinary media.

The protozoa in any soil may occur in the active (trophic) state, or enclosed in cysts. We propose to call the former the "active fauna," and the latter the "cyst fauna"; and we would emphasize the necessity of keeping these two classes clearly distinguished.

Under the varying conditions which obtain in a soil there must be continually changing relations between these two faunas, but at any moment only the members of the active fauna of that period can exert any effect on the soil. To guard against possible misunderstanding, it may be well to state that it is very improbable that the line between the active and the cyst fauna of a soil is one between species and species. There is little doubt that under most conditions a species represented in the active fauna will also be represented in the contemporaneous cyst fauna.

Since the cultural method fails to distinguish between the above two categories, and even leaves unsettled the question of whether an active fauna is present at all, recourse has been made to other methods of examination, which are fully described in the next section. By their aid it has been completely established that an active fauna does exist in a variety of soils ranging from the unmanured plot on Broadbalk field at Rothamsted to sewage-farm soil, leaf mould, and soil from a cucumber border. Some of the results obtained by the examination of these soils will be found in section III.

As regards the forms found, it is improbable that many are generically new; most of them seem to have been described by the older workers on protozoa. Of recent years a very large amount of the literature on protozoa, including the more recent textbooks on protozoology, have been devoted almost exclusively to parasitic forms, so that a worker on soil forms must refer back to the excellent papers of the older authors. References to some of these works will be found in the literature list.

Before the effect of protozoa on the soil can be adequately discussed, it is necessary to gather information about the life led by the protozoan fauna. In particular the effect exercised on the active fauna by the water content, the density of the bacterial flora, the temperature, etc., must be investigated.

Now whilst soil temperatures can readily be determined with sufficient accuracy, the evaluation of the other two factors presents considerable, and in part unsolved, difficulties, which arise largely from the heterogeneity of the soil.

Thus the present method for determining water content deals usually with samples taken to a depth of nine inches. It is clear, however, that if in dry weather a crust has been formed on the surface of the soil, the protozoa may be active at a lower level which might still have a relatively high water content, so that the figure obtained for the water content of the whole soil would give no indication of the actual minimum quantity of water in which protozoa could remain active. This difficulty would be felt even if the soil were a homogeneous mixture; but unfortunately this is far from being the case, and it is certain that in a relatively dry soil the fragments of manure and of decaying plant roots would hold a far larger amount of water than is indicated by an ordinary determination of the water content, so that if, for example, one kilogramme of soil contained 950 grammes of soil particles and 50 grammes of decaying organic matter on which protozoa were flourishing, the figure given by the estimation of the amount of water present in the soil would give no indication as to the actual amount of water in the space where these protozoa were leading an active life.

Another important question is the difference between a coarse grained and a fine grained soil with an equal percentage of water. It would seem quite possible that an active protozoan fauna would be found in the large water spaces in the former at a time when the latter would exhibit no free forms.

Further, when conditions in different soils are to be compared, it is preferable from the biological point of view to express water content as percentage by true volume rather than as percentage by weight.

As regards bacterial counts, all the points which have been urged in connection with the heterogeneous nature of the soil carry here even more weight. In the first place, it is probable that the bacteria are concentrated in groups round decaying organic matter, and it has been found in the examination of fresh films from the soil that the bacteria are present as colonies, and are not scattered singly like currants through a cake. It is obvious that the bacterial count must very largely depend on the degree to which these colonies are broken up during the process of dilution. It is well known, also, that the numbers obtained are dependent upon the medium adopted, and on the conditions of culture.

When the heterogeneity of the soil is taken into consideration, it would seem impossible to hope for an accurate method for the estimation of the active protozoa present in a soil. It is, however, possible that practicable approximate methods may be devised, but before they can be considered satisfactory as a basis for extended experiments, it is very necessary that the range of their probable error should be known.

Up to the present, the only method proposed for the enumeration of the soil fauna is the dilution procedure described by Rahn (11). The work of Cunningham and Löhnis (3) on the thermal death-point of the active, and of the cyst fauna, has been used by Cunningham (4) as the basis of a method of determining the active fauna. He estimates the total fauna of a soil, and, in a second sample, the cyst fauna; the difference between the results is taken as a measure of the active fauna.

Unfortunately, the results obtained by a dilution method will almost certainly be vitiated by the incompleteness of the cultural fauna. As has already been pointed out, present cultural methods fail to indicate, or indicate very late indeed, an important class of soil protozoa, the thecamoebae. Again, the manipulative errors of the successive dilutions, together with the serious risk that shaking will not result in an even distribution of the protozoa through the suspension, introduce a cumulative series of inaccuracies into a troublesome and complicated method. Finally, in common with any other numerical method, it encounters the weighty difficulty of the heterogeneous nature of the soil.

On the whole, therefore, it seems to us that this type of method will be liable to introduce a specious appearance of accuracy into a subject which bristles with difficulties[1].

A very rough, but still valuable, idea of the relative abundance of active protozoa in soils is given, however, by the richness of the fresh fixed films obtained as described on p. 112. In comparing different types of soil only the most striking differences can be regarded as significant, but in considering the variations in the active fauna of one particular soil under changing conditions of temperature, moisture, etc., it is probable that the index of richness of the films obtained will prove a sound basis for general conclusions, although no hope can be entertained of reaching numerical results by this method.

II. METHODS.

It is exceedingly difficult, in an examination of any ordinary soil, to get an adequate idea as to the abundance and nature of the active fauna, and for this reason we have thought it well to describe some of the methods we have found helpful in this work.

By far the simplest method of fixing and staining soil protozoa, whether in cultures or on fresh films from the soil, is by means of cover-slip films. We have usually stored the films in small corked tubes of height 1½″ and diameter 1¼″, and these tubes have been found very convenient for purposes of fixation and staining.

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[1] This criticism does not apply to Cunningham's paper, where it is recognised that precise numbers cannot be given.

If ordinary coverslips are used for this work it is often difficult to decide which side of the coverslip the film is on, particularly if the films have been stored for some time in 70% alcohol. For this reason the coverslips described by one of us in a previous paper ("A note on the protozoa, etc., from sick soils," *Roy. Soc. Proc.*, Vol. 85, 1912, p. 395) will be found very useful. These are oblong coverslips of which one angle has been cut off, and they can be procured from Messrs Frazer, of Edinburgh, Messrs Zeiss, or Messrs Angus. It is obvious that no mistake can arise if it is arranged that the film is always on the lower surface of the coverslip when the long sides point away from the worker and the cut corner separates the right long side from the distant short side.

The methods for the examination of soil protozoa can be divided roughly into three categories, (1) methods for the detection and examination of the active fauna in life, (2) methods for the examination of the active fauna on fresh fixed films of a soil, (3) cultural methods.

(1) *Detection of active fauna in life.* Up to the present we have found no reasonably successful method for the collection and examination of the active fauna of a soil in a living state. Any method which depends upon the addition of water to the soil must admit of very rapid execution, otherwise there is the danger of protective cysts present in the soil opening, and thus giving a false impression as to the constitution of the active fauna. This danger is probably a very real one in the case of the small flagellates, and especially the resting forms of some green algae, in the case of which a few minutes' immersion in water may make the difference between a resting and an active form. Another difficulty seems to be to obtain films adequately rich in comparison with the films got by fixing the fresh soil by the methods described below, and in this respect it is found that methods which give fair results with one type of soil may break down completely with another.

All the methods we have used with any success up to the present depend upon the possibility of collecting and retaining some of the protozoa on a surface film. They all seem uniformly bad, and the only consolation in their use is that the other methods we have tried, including the use of the centrifuge, have up to the present given worse results.

With some rather dry, clay soils, at Rothamsted, fair results were obtained by crumbling a soil into a dish of water, and removing the surface film for the purpose of examination either by floating coverslips on it, or by means of thin wire formed into a circular loop of about ½" diameter.

In the rather coarse, sandy soils, at Abergavenny, fair results were obtained in the case of small flagellates, thecamoebae, and small amoebae, by allowing a stream of water to flow from the tap on to a quantity of the soil in the dish, until the soil was just covered, and then examining the surface films collected as above.

In the case of rather dry, clayey soils at Rothamsted, fairly large amoebae, with a thick pellicle, were obtained by the bubbling process described below.

A glass tube of internal diameter 1½″ and length about 2′ is provided with a singly-bored rubber cork at the lower end. Through this passes a glass tube drawn out to a jet. Connection is made with some form of airblast, so that a stream of air can be blown through the jet. The tube is clamped upright and a newly made suspension in water of the soil to be examined is poûred in until the water level nearly reaches the top. Three hooks (conveniently made of bent strips of "tin") are hung round the rim of the tube in such a way as to furnish a support for the coverslip. The coverslip is placed in position about ¼″ above the water level. Air is now blown through the jet so as to produce a stream of fairly small bubbles rising through the suspension and breaking on the lower surface of the coverslip. The water level can be adjusted within small limits by regulating the air-flow.

After about 30 seconds the air-stream is stopped, and the coverslip lifted off and examined under the microscope. It is frequently of advantage to place a thin sheet of agar jelly on the lower side of the slip before commencing the bubbling, as the protozoa adhere more readily to this substance than to the glass. If this be done, the coverslip is placed for examination, agar side up, on a slide, and another slip is dropped on to the agar surface.

By this method there were obtained from a Rothamsted soil certain amoebae whose presence in the active fauna the other methods had failed to reveal.

Very fair stained preparations of any of the animals obtained by one of the above methods can be made by the ordinary processes in use in the zoological laboratories for making preparations under a coverslip. The easiest method is probably to fix by running a drop of Fleming's solution under the coverslip for a few seconds, then washing through with water, followed by picro-carmine five to ten minutes (this renders the process of staining after the Fleming fixation much easier), washing through again with water, staining with alum carmine for half an hour, washing through again with water, then alcohol up to absolute, followed by terpeneol and balsam. Terpeneol will be found very convenient for this purpose as it clears from a much lower percentage of alcohol than oil of cloves or oil of cedar.

(2) *Examination of active fauna in fresh fixed films.* In the preparation of fresh films from soil to which a fixative has been added we once again depend upon the surface films. For some obscure reason not yet understood, if certain fixatives are added to a quantity of soil a surface film is formed which contains an unknown but probably variable proportion of the active fauna of the soil, cysts, diatoms, moulds, algae, and bacteria. In the production of this result, it is certain that the contained air in the soils. exercises a favourable influence in bringing the animals to the surface film, and really good results cannot be expected by this method from a soil which is absolutely water logged. Of the fixatives we have tried up to the present, picric alcohol (*i.e.*, 50 % saturated solution picric acid in water, plus 50 % rectified spirit), and corrosive alcohol (*i.e.*, 50 % saturated solution corrosive sublimate in water, plus 50 % rectified spirit) have given us the best results.

The best method appears to be to place the soil in a porcelain dish, and pour enough of the fixative through a funnel to the bottom of the soil layer until the soil is just covered. The film so obtained can be taken off on coverslips floated on the surface of the liquid.

Of these two fixatives picric alcohol appears to give richer and more abundant films, particularly as regards small organisms, in sandy soils, whereas corrosive alcohol appears to work better on clay soils, and is more efficient in collecting thick-pellicled amoebae.

The efficiency of the film formation is frequently increased by slightly shaking the dish immediately after the addition of the fixative. The following is a good method for staining and mounting the film so obtained.

Picric Films
Corrosive—2 minutes **Corrosive Films**

70 % alcohol plus a few drops of I_2 in KI	5 minutes
Distilled water	5 minutes
Haemalum	5 minutes
Tap water	Till blue
70 % alcohol	5 minutes
Eosin in absolute alcohol	3 minutes
Absolute alcohol I	1 minute
Absolute alcohol II	1 minute
Xylol I	2 minutes
Xylol II	1 minute

The over-staining in eosin will be found of great assistance in searching rather poor films for active forms, especially in the case of flagellates.

These methods have been found to give very fair results as regards small flagellates, small amoebae, and thecamoebae. Up to the present we have only very rarely found large flagellates and ciliates, but to this question we return in a later part of the paper.

(3) *Cultural Methods.* It would we feel be premature at present to attempt a formal list of the culture media on which soil protozoa flourish. In all cases of cultures of soil protozoa, so far as we are aware, as Vahlkampf clearly insisted in his paper on the biology, etc., of *Amoeba limax*, the protozoa feed upon the bacteria of the culture, and hence almost any culture media on which soil bacteria flourish will probably support a large number of protozoa.

Therefore in those cases in which the expression "pure animal culture" is used we only wish to indicate that the culture contained only one form of protozoon, though of course it contained large numbers of bacteria. It may of course be possible in the future to obtain cultures of some saprozoic protozoa free from bacteria, and in certain cases we have found indications that certain amoebae show a distinct preference for certain culture media, though here, again, this effect may be a secondary one due to the encouragement of a certain type of bacteria.

Up to the present we have mainly used solid media for our cultures, as we find that they are far more convenient for isolating any given form. We used two types of culture media, one an ordinary agar made up of 1000 c.c. meat extract and 15 grm. of agar, but we have found a culture medium of Friedberger and Reiter described in Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, vol. I, gives very good results for most soil protozoa; it consists of a horse-dung agar made up of three lumps of horse dung and 500 c.c. of water, this mixture is boiled for one and a half hours, then filtered through cloth, and finally about 8 grm. of agar is added. In many cases where it is used to get a very strong growth of protozoa it is advisable to add a small amount of water or dilute albumen to the culture plates to about a depth of 2 mm. This addition of water seems to obviate the vacuolated appearance which some workers have noted as characteristic of culture amoebae on plates.

The stock cultures are made up by adding a little soil directly to the plates. If these stock cultures are examined from time to time it will be found that in any given culture there is a more or less definite succession of animal forms. By selecting the time and method of culture it will probably be found possible to get pure animal cultures of any of these forms.

The question how far the dominant active forms in a soil are represented in the cultures depends largely, firstly, on the condition of the soil, and, secondly, on the condition of the cultures. We return to this question below, but it may be pointed out here that in the case of most soils the conditions on the cultures mentioned above seem rather rich for some members of the active fauna, with the result that these forms appear very late in the cultures. A certain check can be obtained on these results by means of cultures in which a small amount of water is added to the soil.

III. SOME RESULTS.

So far the soils which have been examined by the methods described above are relatively few in number, but of varied types.

In three cases, where the soil was taken respectively from a cucumber border, from a fertile garden plot, and from the site of an old manure heap, the soils were probably far richer in farmyard manure than even the most richly manured fields; and correlated with this there was a higher capacity for holding water As would be expected, all the indications were that these soils supported a far denser protozoan fauna than was found in the poorer soils examined.

In the cucumber border, the dominant protozoa were amoebae one of the limax type (*Vahlkampfia soli* n. sp.) and one of the lamellipodian type (*A. cucumis* n. sp.). Thecamoebae, notably a species of Euglypha and a Trinema, could be detected in live films, though they were fairly rare on the fixed films, and were probably the next most numerous protozoa. Flagellates and ciliates were present only in small numbers.

The garden soil, and the soil taken from the site of an old manure heap (both at Abergavenny), contained many amoebae, but a great preponderance of thecamoeban forms. The similarity between their fauna is probably not accidental; it is very likely that the dominance of the thecamoebae in the garden soil was a persistence of the dominance of these protozoa in the manure heap with which the garden had been enriched.

In culture these thecamoebae did not appear in considerable numbers until two or three weeks at least after the culture had been started.

From a consideration of cultural results alone, it would have been imagined that flagellates, both large and small, and amoebae had been the dominant forms.

In a not very rich soil from a cauliflower seedling bed the picric acid method gave a considerable variety of protozoa, no one form of which appeared to have become predominant. It was fairly clear that the density of the fauna was relatively low. It is interesting to observe that this rather poor soil contained many more species than *e.g.* the soil from the cucumber border, though the latter had many more individuals. This suggests an interesting analogy with results obtained on the grass plots at Rothamsted, where the untreated (poor) plot gives a large number of species, whereas on plots which have received a large quantity of manure for many years the number of species is considerably curtailed. A similar phenomenon is shown in rich infusions, in which as a rule at any given moment one or other protozoon has got the upper hand, whilst in ordinary fresh-water pools the fauna is far richer in number of species, but far poorer in number of individuals.

The three Rothamsted field soils (Broadbalk dunged plot, Broadbalk unmanured plot, and a fallow plot on Agdell) also contained protozoa very sparsely, small amoebae being the most numerous, though thecamoebae were also represented. Flagellates were very rare, and ciliates were not found at all in the active state.

In culture, amoebae of the two types found in the cucumber border were prominent, together with a great variety of flagellates and many ciliates. The amoebae on the fresh films seem to be of a type different from either the limax or the lamellipodian amoebae.

Rather large amoebae of two sorts, both with a thick pellicle, were obtained from the dunged plot on Broadbalk (14 tons farmyard manure per acre each year since 1843) by the bubbling method. It is possible that these were more resistant to a comparative degree of drought than the more delicate types which flourished in the wet cucumber soil and came on strongly in cultures from the field soil.

By far the most abundant results were obtained with samples of these soils collected in November, 1913, when the moisture content of the dunged plot was given by the usual method as 22 %. In the dry summer of 1914 when the moisture on this plot varied usually between 13 % and 10 %, very poor results were given by all methods of investigating the active fauna. There is a distinct probability that here the water content is a limiting factor in determining the density of the active fauna.

In the case of the Abergavenny garden soil no clear correlation of this kind was observed; observations were, however, only made in the summer (June) before and after rain.

To get an idea of the fauna of a soil very rich in humus, a deposit of black leaf-mould in a wood near Abergavenny was sampled. Here thecamoebae were again very numerous, amoebae were slightly less numerous, and small flagellates and some ciliates were easily detected. As a further example of a soil rich in organic matter, samples were taken from a sewage bed at Abergavenny. Sewage had been led on to this, and allowed to percolate through. When the samples were taken the bed had dried sufficiently to allow of the deposit being scraped up into heaps ready for removal. Enormous numbers of phytoflagellates (forming a green film on the surface) were present, and thecamoebae and amoebae were very plentiful. Ciliates were not uncommon, and the smaller flagellates were fairly well represented.

As far as these results go, it appears that the numerically most important types of soil protozoa are thecamoebae and amoebae. Flagellates and ciliates are relatively rare. Of the flagellates found, it is very noticeable that the larger forms, such as Bodo and Copromonas and their allies, appear so far to be of very little importance in the active fauna. The most successful soil flagellates are small monads. This is a result which is not revealed by cultural methods, when the larger flagellates assume a much more prominent position. Sherman (14), using a dilution method, found small flagellates to be the most abundant protozoa in the soils with which he worked[1]. Though our observations have not, so far, supported his, we cast no doubt on the substantial accuracy of his results.

The results of examination of the Broadbalk dunged plot in winter and in summer suggest that normal variations in water content may have a considerable effect on the active fauna of the soil, but in the present stage of our investigations we feel it would be premature to lay too much stress on this point.

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[1] Cunningham (4) arrives at a similar result.

IV. CONCLUSIONS.

It seems probable from the work that we have done up to the present that there are always some free living protozoa present in a trophic state in even relatively dry, poor soils.

In manuring on ordinary soil with farmyard manure, a large number of protozoa are introduced into that soil, and if the conditions of culture are such as to necessitate a high water and a high manurial content, the protozoa may well get the upper hand to such an extent as to produce a well-marked deleterious effect on the crop, resulting in the condition known as soil sickness (*e.g.*, in cucumber beds, sewage farms).

The nature of the protozoan fauna seems to vary to a certain extent with the soil under examination. It is probable that this is largely due to actual difference in the fauna of different soils, but it may be partially due to another factor. As is well known, if some soil is added to a hay infusion or other suitable culture medium, the fauna shows a tendency to run in cycles (*e.g.*, at first the dominant forms would be found to be small flagellates; these are usually followed by larger flagellates and amoebae, and these are succeeded by ciliates). It is possible that such cycles may occur in the soil, and it is possible therefore that two soils with a similar water content may show quite different active fauna, depending on the point of the animal cycle at which that soil had arrived. The dominant protozoa found in a trophic state in a soil may be the dominant form found in the cultures, as was probably the case in some sick cucumber soils; but it of course depends on the suitability of the medium, and the culture method adopted. It is probable that the richer the soil and the higher the water content at the time of examination, the greater the probability of the dominant culture form being the dominant trophic form in the fresh soil. A possible exception to this rule is furnished by the thecamoebae, which usually only appear late under present cultural conditions.

It will be seen that up to the present the dominant active fauna of the soil, as shown by the fresh films, consists mostly of amoebae, thecamoebae and small flagellates.

In this connection there is one point which requires further investigation, and that is the frequent prevalence of relatively large flagellates in soil cultures (*e.g.*, Prowazekia and Copromonas), whereas in fresh films the only flagellates found are very small monads. It may perhaps be found that the Prowazekia are present in the trophic state only in groups on the decaying organic matter in the soil, possibly only for short periods, and that the encysted forms present in the soil are favoured by the condition of the culture at the expense of the smaller flagellate forms, or it is possible that these large flagellates are contented with a very short trophic life in the soil at a time when the water content is high and there are large quantities of decaying material in the soil.

Under these conditions it is not unlikely that the ciliates so frequently found in soil cultures lead a trophic life in the soil.

There is another factor which must be reckoned with in this connection, and that is the possibility that the present methods for the examination of fresh soil films do not give a fair account in regard to these large flagellates, which may be caught up by their flagella amongst the soil particles.

None of these possibilities is mutually exclusive, and it seems from recent work on cultures of soil to which water alone has been added that the last explanation is not very probable.

In conclusion, it seems to us that there are three categories under which the protozoan population of any soil at a given moment should be studied, (1) the active fauna, (2) the resting fauna (in cysts), and (3) the cultural fauna. In the immediate future better methods must be devised for the detection of the active fauna, a complete study is needed of the possible seasonal variations which might result in a transfer of certain forms from the resting fauna to the active fauna, and a more careful study must be made of cultural conditions, so that it may be possible to cultivate at once any desired member of the active fauna of a soil.

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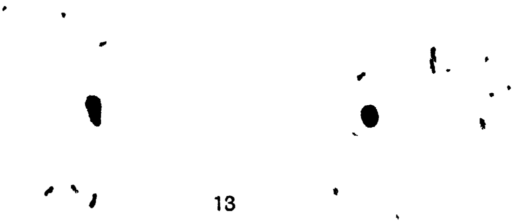

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DESCRIPTION OF PLATES.

PLATE II.

FIG.

1. *Euglypha* sp. from fresh fixed film (see p. 112) of cucumber bed. A thecamoeba.
2. *Chiloden* sp. from fresh fixed film of cucumber bed. A ciliate.
3. Flagellate from fresh fixed film of cucumber bed.
4. Dividing *Vahlkampfia soli* from fresh fixed film of cucumber bed. A limax amoeba.
5. *Euglypha* sp. from fresh fixed film of cucumber bed. A thecamoeba.
6. *Chlamydophrys* sp. from fresh fixed film of cucumber seedling bed. A thecamoeba.
7. *Amoeba gobanniensis* from fresh fixed film of cucumber seedling bed. A lamellipodian type of amoeba.
8. *Amoeba* sp. Do.
9. *Amoeba* sp. Do.

PLATE III.

10. *Vahlkampfia soli* from fresh fixed film of cucumber bed. A limax amoeba.
11. *Vahlkampfia soli* stage in division.
12. *Amoeba cucumis* from young culture. A lamellipodian amoeba.
13. *Amoeba cucumis* late stage in division.
14. *Bodo caudatus* from a culture. A flagellate.
15. *Bodo caudatus* stage in multiple division.

Note. These illustrations are designed to assist bacteriologists and others who are interested in soil protozoology to refer the species they will encounter to the general type. It is hoped in particular that the organisms vaguely referred to as "Amoebae" may be more definitely distinguished at least into Thecamoeba and Amoeba. The limax and the lamellipodian type of amoebae will almost certainly be among the most successful amoebae found in cultures, and it would be of interest to distinguish them from one another and from other less defined types. The sizes of the protozoa shown varied from 15 to 50 μ; but the figures were not drawn to the same magnification.

(*Received December* 21*st,* 1914.)

SOME CONSIDERATIONS AFFECTING THE GROWING OF LINSEED AS A FARM CROP IN ENGLAND.

I. VARIATIONS IN THE OIL CONTENT.

By J. VARGAS EYRE, M.A., Ph.D. and E. A. FISHER, M.A. (Oxon.).

(Research Department, South Eastern Agricultural College, Wye, Kent.)

(With 2 Text-figures.)

Owing to the rapid rise in the price of linseed and linseed products during recent years, the question has arisen as to whether the farmer can grow linseed, for home consumption, at a smaller cost than he can buy it for under existing conditions. This question involves considerations as to which of the several kinds of linseed would be the most profitable to grow, and it becomes necessary to consider not only the agricultural requirements of the crop[1], but also the fact that the value of this crop is largely, if not entirely, determined by the quantity of oil produced per acre.

Numerous experiments on quite a small scale have been carried out at different times in different parts of the country and, notwithstanding much discordancy, the general indication seems to be in support of the opinion that given the most suitable variety and an average season, linseed growing in this country is a profitable undertaking.

In some parts of the world flax is grown for seed only—*i.e.* as *linseed*[2] —while in other parts it is grown for fibre (seed being a secondary consideration)—*i.e.* as a *line*[3] crop, and the belief has gained general credence, that high quality fibre alone could be obtained combined with inferior seed—inferior, that is, from a feeding point of view. The work of Ivanoff[1] however goes to prove that this view is erroneous and that there is little difference in oil content between the seed from the fibre crop, *i.e. flax seed*, and that from the linseed crop; and this has been confirmed by the present writers.

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[1] "Notes on Linseed. I. Linseed as a Farm Crop," *Journ. South Eastern Agric. College*, Wye, 1914.

[2] *Vide* "The Growing of Linseed for Feeding Purposes," *Journ. Bd. of Agric.* 1913, **xx.** pp. 377–385.

[3] *Vide* J. V. Eyre, *Science Progress*, April 1913, pp. 596–628, Supplement to *Journ. Bd. of Agric.* No. 12, Jan. 1914, and *Journ. Roy. Agric. Soc. England*, 1913, **74**, pp. 127–141.

Our results indicate that the only difference between the seed from the fibre crop and that from the linseed crop is one of yield, there being little difference in oil content between the two kinds. This is important because it indicates that by harvesting early the best quality fibre may be obtained without materially lessening the oil content of the seed and consequently the value of that commodity. For the only rational way of valuing linseed is on its oil content; oil per acre from the farmer's point of view; oil per ton from the factor's point of view. In consequence of the increased demand for linseed oil it cannot be emphasised too strongly that both farmer and factor must sell or buy linseed on an oil basis and the farmer would do well to realise that this is the only possible basis on which he can sell his crop; that he is, in other words, growing oil rather than seed.

The main object of the present communication is to point out the varieties of linseed best suited to English conditions and giving the largest yield of oil per acre, and also to ascertain the effect of artificial manures on the oil content of the crop produced.

The possibility of harvesting linseed of high oil content from a *line* crop becomes apparent from Ivanoff's work already referred to on the oil-forming processes occurring in oil-bearing seeds. Ivanoff developed the subject from the plant physiologist's point of view and did not concern himself with the agricultural or economic aspect, but his work is nevertheless of considerable interest agriculturally. He estimated the oil present in the linseed at different periods after flowering and his results for one series of determinations with a crop grown in 1910 are shown in the accompanying table and graph.

An examination of these results shows that for the first two weeks after flowering the seed material contains very little oil. After this comes another period of two weeks during which the formation of oil takes place with great rapidity. During the last two or three weeks the increase in oil content was found to be extremely small, amounting to only about 2·5 % increase in three weeks, whereas the increase during the previous two weeks amounted to more than 21 %. The optimum oil formation, therefore, occurs exactly in the middle of the period between flowering and final ripening, and the results seem to indicate that, from this point of view, little is gained by allowing the seed to ripen before harvesting the crop. In view of its agricultural interest an attempt was made by the present authors to repeat this work. As no mention is made by Ivanoff as to the means adopted to secure seed of uniform ripeness at the dates of gathering and as difficulty was experienced in doing this a different procedure was adopted. Instead of gathering the seed at different dates after flowering, the seed collected at harvest was separated into four groups of increasing degrees of ripeness, viz.:

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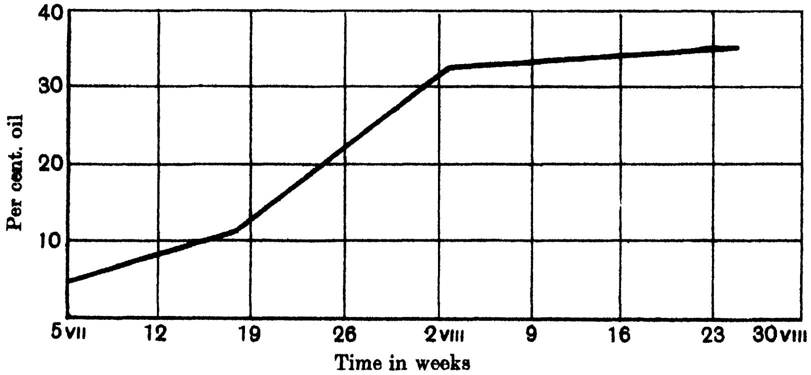
[1] *Beihefte zum Bot. Centrb.* Band XXVIII. Heft 1, pp. 159–191 (Jan. 1912).

	I	II	III	IV
Harvested	1st week after flowering July 5th	July 18th	August 3rd	August 25th
Percentage oil	4·37	11·00	32·50	35·04



A. The seed quite green;
B. The seed just beginning to turn brown;
C. The seed wholly brown, but not loose in capsule;
D. The seed fully ripe, *i.e.* quite loose in capsule.

Determinations of the oil content of these various groups gave the following results:

A. 19·86 %; 22·23 %; mean = 21·045 %;
B. 25·30 %; 29·17 %; 35·77 %; mean = 30·08 %;
C. 37·41 %; 38·94 %; mean = 38·03 %;
D. 40·84 %; 40·93 %; mean = 40·88 %.

Notwithstanding the fact that, in all cases, the analyses were made immediately after gathering the seed, as had been anticipated, it was not found possible to obtain concordant results with the less ripe samples although such was not the case with the fully ripe seed. Presumably this is due to the rapidity with which oil is accumulated in the seed during the earlier stages of ripening—during the period represented by the more rapidly rising portion of Ivanoff's graph. During this period of development a slight difference in maturity would have a relatively large effect on the oil content and this is indicated by the different results obtained with the three samples of seed in group B. During the later stages of development, in which the oil forming processes slow up, more concordant results were obtained, as exemplified by group C, while in the final stages oil formation has almost ceased and consequently the oil content remains practically constant.

It is evident that, taken generally, these results are quite in harmony with those of Ivanoff.

Further evidence on this point is afforded by the fact that whenever imported *flax* seed is grown in this country as a *linseed* crop the oil content of the seed obtained invariably approximates to that of the parent seed; and this in spite of the fact that in the latter case the seed is allowed to ripen before the crop is harvested, and in the former case the crop is harvested in an unripe condition. Thus a sample of white flowering Dutch seed, grown in Holland as a flax crop, and pulled early, the seeds being allowed to ripen in the stook, contained 35·49 % of oil. This sample, grown at Wye in 1913 as a linseed crop, gave seed containing 35·08 % oil; and that grown at Harper-Adams Agricultural College in Shropshire had 36·71 % of oil.

Comparison of English grown and imported linseed.

It has been maintained by some writers that English grown linseed is inferior to that grown in the better known linseed growing countries. Thus J. A. Voelcker[1] states that although English grown linseed stands very fairly as regards oil content, it is neither superior nor equal to the best grown foreign seed and in support of this contention quotes the following figures which were obtained from various sources:

Black Sea	38·4 %	English grown	36·7 %
Bombay	38·2 ,,		33·5 ,,
St Petersburg	35·3 ,,		32·8 ,,
Alexandria	35·7 ,,		
Riga	34·7 ,,		

Further he stated that he had been unable to find any instance of English grown linseed that was better than the foreign linseed, and

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[1] *Journal of Farmers' Club*, 1897, p. 65.

"the average, as one would naturally expect in a climate such as ours, must be considerably below what is produced in India, and in countries where the climate is favourable to the production of oil." As the figures quoted below will indicate, such a view is entirely opposed to our own experience.

In the first place it is necessary to point out that the figures quoted by Voelcker do not constitute a real comparison since he says nothing of the nature or source of the English grown seed: one cannot compare an imported Bombay seed with an English grown Dutch, and there is no indication that this is not done in the examples quoted. And then, too, different samples of the same variety grown in the same region in different seasons show wide differences in oil content—differences that are often far greater than any there may be between English grown and imported seed. This is well illustrated in the following table:

	J A Voelcker[1]	A. Voelcker[2]	Leather[3]
Black Sea	38·4 %	30·78 %	—
Riga	34·7	31·19	—
Bombay	38·21	—	40·71 %

The only method of obtaining a strict comparison is by comparing samples of the different varieties grown in different parts of the world with the seed produced when the *same samples* are sown in different parts of England. Such a comparison should be carried out during several successive seasons. This is being done: linseed trials are being conducted in accordance with a scheme drawn up by the British Flax and Hemp Growers' Society, Ltd. on the College farm at Wye; at Camblesforth Hall farm in Yorkshire; at the Seale-Hayne Agricultural College in Devonshire; at the Harper-Adams Agricultural College in Shropshire; and at the Holmes Chapel Agricultural College manurial trials are being carried out. Below are given the results for the season 1913, and we hope to continue the work over several years so as to make the comparison a stricter one by eliminating the effect of seasonal variations. It is worthy of note when considering the following observations that the season 1913 was an average one as regards weather and was not specially favourable to the linseed crop, either as regards yield or oil-formation.

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[1] *Journal of Farmers' Club*, 1897, p 65.

[2] "On the characters of pure and mixed Linseed Cakes," *Jour. Roy. Agric. Society*, 1873, pp 1–51.

[3] "The Comp. of Oil seeds of India," *Mem. of Indian Dept. of Agric.* (Chem. Series), Vol. I. (1907), No. 2, pp. 13–38.

The method employed in estimating the oil content was the ordinary ether extraction method and requires but a brief description. Each sample of seed was carefully examined and only sound seeds were used in the estimation. Damaged or otherwise unsound seeds were rejected as was also adventitious matter such as weed-seeds, chaff, etc. About

TABLE I. *Showing Oil Content of English grown and Imported Linseed.*

Variety of Seed	Imported 1912	English grown 1913	
Pskoff (Imported direct)	37·45 %	36·68 %	(Selby)
Moroccan—Mazagan (London market)	40·60	42·90 40·13 39·06 40·86	(Wye) (Camblesforth) (Seale-Hayne) (Harper-Adams)
Plate (London market)	38·45	42·80 39·69 37·72 41·35	(Wye) (Camblesforth) (Seale-Hayne) (Harper-Adams)
Dutch—white flowering (London market)	35·49	37·69 35·08 34·60 36·71 34·08	(Wye) (Camblesforth) (Seale-Hayne) (Harper-Adams) (Holmes-Chapel)
Dutch—white flowering (Imported Gröningen)	38·65	38·30	(Selby, as line crop)
Dutch—Riga child (Imported Rotterdam)	36·13	37·11	(Selby, as line crop)
Steppe—Russian (Liverpool market)	38·90	41·50	(Wye)

Note. The amount of moisture present in the seeds was not determined but an examination of many published figures showed this to be never less than 5·5 % nor above 11 %; while in by far the greater number of cases the amount was between 7 % and 9 %. The variations seem to be largely due to climatic conditions; the hotter the climate the smaller the moisture content.

On the other hand, the constancy in the water content of linseed grown in different parts of the same country under roughly comparable climatic conditions is really remarkable. Leather gives the following for Indian varieties:

Punjab	7·60 %	(Average of 10 samples)
Central Provinces	6·73 %	(„ 21 „)
Bombay Presidency	6·81 %	(„ 7 „)
Madras Presidency	6·72 %	(„ 5 „)

In view of these facts it seemed to us redundant to estimate systematically the moisture present in the seeds we have used because such small variations could not mask (and in some cases would tend rather to increase) the difference in oil content indicated in the table.

5 grms. of the seed was thoroughly ground in a mortar with silver sand which had been extracted with boiling hydrochloric acid and washed free from acid and soluble salts. The finely ground material was transferred to a Soxhlet extraction thimble; anything remaining in the mortar being washed into the thimble with petroleum ether (B.P. below 40° C.). The extraction was carried out in a Soxhlet Extraction Apparatus with petroleum ether and was continued for some 20 to 24 hours. The ethereal extract was then filtered into a weighed flask and the ether distilled off on a water bath and the oil freed from any remaining ether and dried in a steam oven at 98–99° C. Every half hour the flask was cooled in a desiccator and weighed. The series of weighings thus obtained showed first a decrease as ether and moisture were expelled, and then an increase as the oil slowly oxidised. The smallest weight was taken as the true weight of the dry oil. Duplicates were carried out in all cases and invariably were in close agreement; the greater number being well within 0·25 %.

The figures given (Table I) indicate that linseed grown in England is by no means inferior in oil to any of the imported samples. Indeed, the results as a whole compare by no means unfavourably with the figures given by Leather[1] for 52 samples of Indian linseed grown in all parts of India, in spite of the fact that the climate of India is generally considered to be especially favourable to the growth of oil-bearing crops.

Summary of Leather's results for Indian Linseed.

District	Aver. % of oil	Varying between
Punjab	38·27 (aver. of 10)	35·6 % and 41·91 %
Central Provinces	41·36 (,, 21)	36·47 ,, 44·20
Bombay Presidency	40·71 (,, 7)	41·23 ,, 44·45
Madras Presidency	40·12 (,, 5)	40·46 ,, 41·71
United Provinces	42·58 (,, 9)	41·44 ,, 44·55

The crops grown at Wye gave consistently higher figures than those at the other centres, from which it would appear that linseed is more suitable as a south country crop, doing best under the warmer more equable weather conditions of our southern shores. If this is so, one would expect similar high figures for the linseed grown at the Seale-Hayne Agricultural College (the Devonshire Centre). Here, however, the season was a very wet one, the yield was low (in one case less than a hundred-weight to the acre), and great difficulty was experienced in harvesting and drying the crop. The seed was black and of poor quality and had a distinct "musty" odour; and it was a matter of some surprise that the oil content was as high as it was actually found to be.

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[1] Leather, *loc. cit.*

Variety to Sow.

Many so-called varieties of linseed are cultivated at the present day and they exhibit differences sufficiently well marked for them to be classified by some authorities as varieties of different species. It is probable that in some cases they are not real botanical varieties at all but rather "economic" ones brought about by long continued cultivation in different climates and different methods of treatment. These differences, however, persist for a reasonable period when the crop is grown away from its natural environment, and this being so, linseed grown in or exported from any particular region is generally called a variety if it shows any well marked and fairly persistent characteristic.

In deciding which are the most profitable varieties to grow, not only the percentage of oil. but also the yield per acre must be taken into account. In other words we should be able to express the return of oil per acre before we can effect a strict comparison between the different varieties. And this comparison should be made between the more commonly grown varieties in as many different localities as possible and during several seasons before any certain conclusions can be drawn. In the present communication we have endeavoured to obtain some information on the point in the case of those varieties which were grown in 1913 at four of the Centres already mentioned (*vide* Tables II A and II B). By multiplying the percentages of oil in the samples by the yields in cwts. per acre and dividing the products by 100 we obtain the yields of oil per acre, and the numbers so obtained afford some indication of the relative merits of the different varieties dealt with. Considering the wide variations in yield and the great differences in the quality of the seed grown at the different centres, the results as set forth in the accompanying tables are remarkably consistent and bring out the relative merits of the different varieties in a very striking manner. Plate seed comes an easy first, Steppe seed a moderate second, Moroccan third, while Dutch is the poorest of the four; except in the case of the Harper-Adams crops of which the Plate and Moroccan varieties do not appear to have done as well as was the case at Wye and Camblesforth. It is noticeable that even the very poor crops from the Seale-Hayne centre still indicated the marked superiority of the Plate seed over the other varieties as an oil-producing crop.

TABLE II A.

Plot No.		Type of sowing	Yield, cwts per acre	Average yield thickly sown plots	Average yield thinly sown plots	Total average yield	Percentage oil	Yield of oil per acre, percentage oil × yield ÷ 100	Oil per acre when Plate = 100	Relative order
Moroccan	10*	thick†	12 cwt. 20 lbs.	14 cwt. 44 lbs.		12 cwt. 25 lbs.	42·9	6·1775 cwt.	62·5	3
	15‡	„	16 „ 64 „					5·2435 „	83·2	3
	11*	thin§	8 „ 16 „		10 cwt. 8 lbs.			4·3200 „	98·5	2
	13‡	„	12 „ 0 „							
Dutch	1*	thick	11 „ 2 „	12 cwt. 46 lbs.		11 cwt. 86 lbs.	37·7	4·6785 „	62·3	4
	7‡	„	13 „ 68 „					4·0515 „	74·6	4
	3‡	thin	13 „ 12 „		11 cwt. 14 lbs.			4·1920 „	66·9	4
	4*	„	9 „ 16 „							
Plate	9*	thick	14 „ 64 „	14 cwt. 72 lbs.		14 cwt. 70 lbs.	42·8	6·2670 „	100	1
	16‡	„	14 „ 80 „					6·2595 „	100	1
	12*	thin	10 „ 0 „		14 cwt. 70 lbs.			6·2575 „	100	1
	14‡	„	19 „ 24 „							
Steppe	6*	thick	11 „ 40 „	13 cwt. 4 lbs.		13 cwt. 53 lbs.	41·5	5·4095 „	74·2	3
	8‡	„	14 „ 80 „					5·6150 „	89·7	2
	2*	thin	13 „ 4 „		13 cwt. 98 lbs.			5·7685 „	92·2	2
	5‡	„	14 „ 88 „							

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* These plots were very poor chalk soil; plots 6 and 12 being especially poor. On these plots the crop was noticeably poor and thin.

† *i.e.* 112 lbs. per acre.

‡ These plots were alluvium and had been prepared for potatoes and were in a better and more sheltered situation than the chalk plots. The differences in soil and situation may possibly account for the anomalies which are noticeable in the yields from the different plots. This will be dealt with at length in a "Report on Linseed Experiments for 1913."

§ *i.e.* 70 lbs. per acre.

TABLE II B.

Variety	Yield		Per cent. oil	Oil per acre	Oil per acre (Plate = 100)	Relative order
Camblesforth	cwt.	lbs.				
Plate	6	12	39·69	2·423 cwt.	100·00	1
Moroccan	5	67	40·13	2·247 ,,	92·45	2
Dutch	4	74	35·08	1·635 ,,	84·93	3
Seale-Hayne						
Plate	3	63	37·72	1·345 ,,	100·00	1
Moroccan	1	21	39·06	3·977 ,,	29·55	2
Dutch	0	106	34·60	3·274 ,,	24·33	3
Harper-Adams						
Plate	8	97	41·35	3·667 ,,	100·00	2
Moroccan	8	67	40·86	3·513 ,,	95·80	3
Dutch	9	74	36·71	4·465 ,,	121·76	1

Relation between oil content and size of seed.

Leather[1] when examining Indian linseeds, states that he could get little evidence that any connection exists between the size of the seed and the percentage of oil they contain. Some of the large kinds from the Central Provinces weighing 8·0 grm. per 1000 seeds were found to contain only as much oil as one grown at Bilaspur (in the Central Provinces) which weighed only 5·5 grm. per 1000 seeds. His comparisons, however, are not strict ones, for the Central Provinces and the other regions of India from which he obtained his seeds are vast areas, over which very varied climatic conditions obtain, and in many cases they produce types of linseed exhibiting well marked characteristics. It is highly probable that in these instances any correlation of size of seed with oil content would be hidden by the far greater variations due to variety and varying climatic conditions under which they were grown. That this may be so is evident from a comparison of the oil content and size of seed grown in different parts of Bombay Presidency, a region throughout which the climate is more uniform than is the case with the other regions of India.

Weight of 1000 seeds	Oil content
6 to 7 grm.	41·23 % (single sample)
7 to 8 grm.	42·22 % (average of 3 samples)
8 to 9 grm.	43·83 ,, ,, ,, ,, .

These show a distinct, though slight, increase of oil content with increase in size of seed. Our own experience with both English grown and imported seed goes to support such a relationship. From Table III it will be seen that by comparing either the imported or the home grown varieties with one another, very little regularity can be observed. It is evident that in this case the variations in oil content due to difference of variety mask any difference due to varying size of seed. Very different, however, is the case when we compare each imported variety with the seed produced *from the same sample* grown in England. Here a regularity is noticeable: in practically all cases an increase in oil content is accompanied by an increase in size of seed. Strict proportionality between the two could not of course be expected from the very nature of the case, but that there is a parallelism between them, other things being equal, seems to be sufficiently brought out by the figures given in the table below.

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[1] *Loc. cit.*

TABLE III. *Showing Relation between Oil Content and size of Seed of Different Varieties of Linseed Grown under Different Conditions.*

Variety of seed	Imported		English grown	
	Oil content	Wt. of 1000 seeds	Oil content	Wt. of 1000 seeds
Pakoff	37·45 %	4·198 grms.	40·55 %	4·484 grms.
Moroccan	40·60	10·166 ,,	42·90 40·13 39·06 40·86	13·098 ,, 13·538 ,, 11·132 ,, 13·392 ,,
Plate	38·45	6·108 ,,	42·80 39·69 37·72 41·35	8·840 ,, 9·204 ,, 7·712 ,, 8·744 ,,
Dutch	35·49	4·817 ,,	37·69 35·08 36·71 34·60 34·08	5·410 ,, 4·810 ,, 5·164 ,, 4·066 ,, 3·904 ,,
Dutch	38·65	4·754 ,,	38·30	4·861 ,,
Dutch	36·13	4·599 ,,	37·11	5·252 ,,
Steppe	38·90	5·076 ,,	41·50	7·198 ,,

Frequent Change of Seed.

In Russia, the country from which the best flax seed is obtainable, change of seed is not an agricultural consideration. The crops are almost invariably grown from seed of the previous harvest and in many cases the farmers have had their seed in the family for more than 20 years[1].

In all other European countries, however, emphasis is laid upon the necessity of frequently changing *flax seed*, and the same practice has been recommended in the case of linseed. It is not known definitely, however, whether continuous growing from seed of the previous year's crop has any effect on the oil content of the seed and in this connection very few data are available. In one case[2] Riga seed was sown in Essex in 1911 and the oil content of the resulting seed was 35·66 %. The seed from this crop was sown in 1912 when it produced seed containing only 26·73 % of oil. Such a decrease (nearly 9 per cent.) is, however, very unusual whatever the cultural conditions may be and it is probably due to some other cause than the one in question. Personally, we have never come across any sample of seed with such a low oil content as this; the lowest we have met with was in a sample discovered by one of us growing wild in the south of Ireland and consisting of very small seed with a particularly hard and thick seed coat. This had an oil content of 29·07 %.

The only other data available, as far as we have been able to discover, are some given by Leather. He grew specimens of linseed rich in oil, obtained from various parts of India, at Lyallpur in the Punjab at farms where seed of poor quality was generally produced and, as the subjoined table shows, found a small but continuous decrease in oil throughout two years.

	Orig. seed (1904)	Produce of 1905	Produce of 1906
White Linseed from Cawnpore	44·62 %	41·28 %	39·90 %
,, ,, ,, Khandwa	44 96	44·18	42·93
,, ,, ,, Damoh	45 34	43·07	43·57
Brown ,, ,, Partabgarh	43 17	40·98	38 31
,, ,, ,, Cawnpore	42·05	40·97	39·43
,, ,, ,, Sholapun	41·13	40 42	38·82

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[1] J. V. Eyre, Sup. to *Journal Bd. of Agric.* No 12, Jan. 1914, p. 17.

[2] *Journal Bd. of Agric.* Vol. xx, No. 5 (Aug 1913), p. 381.

In this case, however, the crops were grown on farms which produced poor quality linseed only and hence the conditions were all in favour of a decrease in oil content.

Up to the present we have not had the opportunity of testing this in many cases, but a few data have been obtained with Pskoff and Plate seed grown for several successive generations in different parts of England.

The results are given in Table IV.

TABLE IV.

Pskoff seed.	Imported 1911	37 45	% oil
,,	Grown Wimbledon (1912) from 1911 imported seed	35·65	,,
,,	Grown Wye (1913) from Wimbledon 1912 seed	33·21	,,
,,	Grown Wye (1914) from Wye 1913 Wimbledon 1912 seed	33 35	,,
,,	Grown Wye (1913) from imported (1911) seed	34·05	,,
,,	Grown Wye (1914) from Wye 1913 seed	34·96	,,
,,	Grown Wye (1914) from imported (1911) seed	33·78	,,
,,	Grown Selby (1913) from imported (1912) seed	36 68	,,
,,	Grown Selby (1914) from Selby (1913) seed	35 05	,,
Plate seed	Imported (1912)	38 45	,,
,,	Grown Wye (1913) from imported (1912) seed	42 82	,,
,,	Grown Wye (1914) from Wye (1913) seed	41 64	,,
,,	Grown Wye (1914) from imported (1913) seed	39 10	,,

It will be seen that in some instances a diminution in oil content does occur from generation to generation and there are indications that the percentage of oil produced eventually becomes more or less constant. On the other hand such diminution might conceivably be due to seasonal and cultural variations or to variations in soil factors from year to year.

Such effects could be eliminated by growing parent, child, grandchild, and great-grandchild etc seed during the same season and on the same plots. This has been done in one or two instances and as shown in the table the variations then disappear; practically no difference in oil content being exhibited by the various generations when grown side by side in the same season and under identical conditions of soil and cultivation.

At any rate our results give no support to the view that repeated growth of linseed from the same stock gives rise to a seed of diminished oil content.

The effect of artificial manures on the oil content.

The use of artificial manures has been found to occasion only a very slight variation in the oil content of linseed. This conclusion is based upon the results of trials carried out with Dutch seeds at Rothamsted during the hot season of 1911 and at Holmes Chapel Agricultural

College during 1913 (cf. Table V). As will be seen from the table in no case was there a difference in the total oil content of more than 1·6 %: a difference which is of but slight economic importance. The main effect produced by the artificial manures has been found to be in the direction of influencing the yields both of seed and straw as may be seen from the following illustration

TABLE V. *Showing effect of Manuring on the Oil Content of Linseed.* (Dutch white flowering.)

Holmes Chapel				Rothamsted—Hoos barley field		
Plot No.	Type of sowing	Manuring	Oil content	Plot No.	Manuring	Oil content
1	Thin	none	33·22 %	1 A	N	34·45 %
2	,,	N	33·57	2 A	N + P	34 11
3	,,	N + P	33·45	3 A	N + K	34 25
4	,,	N + P + K	34·08	4 A	N + P + K	34·67
5	Thick	none	34·47			
6	,,	N	34·79			
7	,,	N + P	34·35			
8	,,	N + P + K	34 53			

Dutch white flowering linseed, grown at Rothamsted

It has been found at some centres that it is the combination of superphosphate and potash that brings about the most striking differences of yields; and it is interesting in this connection that the only significant increase in oil content due to manurial treatment is brought about by the same combination. At other centres owing, presumably, to unfavourable condition of experiment the application of artificial manures seems to have had little or no effect in increasing the yield of the crop. This point, however, cannot be dealt with here but, together with other considerations of a purely cultural nature, is reserved for a separate report.

(*Received January* 14*th*, 1915.)

VOLUME VII SEPTEMBER, 1915 PART II

THE EFFECT OF CLIMATE ON SOIL FORMATION.

BY J. WALTER LEATHER, V.D., F.I.C.

Imperial Agricultural Chemist, Agricultural Research Institute, Pusa, Bengal.

IN a recent volume[1] of the Journal of the Royal Agricultural Society of England, Russell deals with some of the effects of climate and weather on soils.

In the opening paragraphs the effect of climate on the formation of "the mineral framework of the soil" is alluded to and the laterite of the Tropics is mentioned as an example of a soil produced "under wholly different conditions" of climate, *i.e.* different from the climate of Europe.

There are two soils in India whose mode of formation is still unexplained. The laterite is one and the other is the Regur or Black Cotton Soil, the latter occupying very much the larger area. These two soils are very distinct in their several chief characteristics.

The Regur is black or dark brown in colour; when wetted it expands to an unusual degree and on drying large fissures form in it; it retains unusually large amounts of water; it is practically devoid of stones except where near rock formations; in chemical composition it frequently contains 5 to 10 per cent. of calcium carbonate, though also the proportion of this constituent sometimes falls to less than 1 per cent. It rests on various rock formations; a large part of the area rests on the Deccan trap, but it is also found on metamorphic rocks in Southern India and on the Cuddapah quartzites.

When laterite rock is first exposed it is soft and light coloured, but it rapidly hardens on exposure and after breaking down to soil is red; it frequently or generally contains gravel, including limonite; but the soil has otherwise no very special physical features. Like the Regur however it is found to pass abruptly into very various formations, trap, metamorphic, sedimentary. In fact the association is remarkably alike in the two cases.

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[1] Vol. LXXIV. p. 1, 1913.

Some years ago Holland[1] drew attention to the fact that laterite is largely not a silicate but a hydroxide of aluminium, and pointed to the difficulty of explaining its formation on chemical grounds and suggested that this might be attributed to organic life with greater probability. This suggestion has not I think been followed up.

In a recent Memoir Harrison and Ramaswamy Sivan[2] have detailed the characteristics of the material which is largely responsible for the black colour of Regur, and have shown that it is a colloidal material containing iron, aluminium and silica associated with a small proportion of organic matter and is not attacked by concentrated hydrochloric acid. The circumstances in the neighbourhood of a bed of Regur are peculiar, for at one place one can see the rock taking on the dark colour of Regur, at another not far away the same rock is weathering to red soil. It is quite possible that here too we have an instance of bacterial action.

In any case the formation of these two soils cannot be simply attributed to either weather or climate. It occurred to me therefore that the above facts might be of interest to those who are concerned with the biology of soils.

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[1] *Geolog. Mag.* Vol. x (1903), p. 59.

[2] *Memoir Dept. Agri. Ind*, Chem. Ser. Vol II. No. 5.

(*Received August 20th,* 1914.)

PROBABLE ERROR IN PIG-FEEDING TRIALS.

By CHARLES CROWTHER, M.A., Ph.D.

Department of Agriculture, University of Leeds.

In an earlier issue of this *Journal*[1], Robinson and Halnan have communicated the results of a statistical analysis of three sets of pig-feeding experiments from which they conclude that "the probable error of one animal in a pig-feeding experiment is in the region of 10 per cent. of the average live-weight increase."

The whole subject of the interpretation of the results of feeding experiments has since been exhaustively reviewed by Mitchell and Grindley[2]. From the results of 17 American experiments on swine, involving 507 pigs divided into 49 lots of 5 to 23 pigs each, of initial weights ranging from 24·7 lbs. to 354·4 lbs., and feeding periods ranging from 55 to 126 days, they arrive at a figure (11½ per cent.) for the probable error which is substantially in agreement with the above. The range of probable errors for the separate lots in all the experiments quoted above is from 2½ to 33 per cent. of the live-weight increase.

In an earlier discussion of the subject of the experimental error in feeding experiments Wood and Stratton[3] record an experiment with four steers of similar age, weight and past history. Their live-weight increases for a period of ten months, during which they received identical feeding and general treatment, were so closely in agreement that the probable error of any one of the four animals was only about 0·5 per cent. of their average increase. Nevertheless, when put on to a fattening ration they gave divergent results and "after three months' feeding their probable error was as usual 13 per cent. of their average live-weight increase." They conclude therefore that the experimental error cannot be appreciably reduced by such careful selection of the animals and that "the requisite precision in feeding trials can only be obtained by increase of numbers, or, if that is impossible, repetition of the experiment." This conclusion is criticised by Mitchell and Grindley as being "not based upon sound and sufficient evidence" and not in harmony with the results of their own analysis of more comprehensive data. They quote, in particular, the results of three Canadian experiments with swine, comprising 10 lots of 5 to 10 pigs each, in which the probable errors are remarkably, and, with one exception (13·5 per cent.), uniformly low, averaging only 7·4 per cent. The probable errors of the separate lots, excluding the highest, ranged from 2·5 to 10·4 per cent. "The reports of these experiments are too meagre to enable one to tell what feature or features of experimental control are responsible for this low variability."

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[1] This *Journal*, Vol. v. p. 48.

[2] University of Illinois Agric. Exp. Sta. Bulletin No. 165 (July, 1913).

[3] This *Journal*, Vol. III. p. 417.

The case of the pig is clearly one for special consideration in this connection, since its more prolific breeding qualities enable a much closer equalisation of experimental lots with regard to breed and age than is possible with cattle and sheep. This is notably of advantage as regards breed, since this factor undoubtedly contributes more than any other to the disturbing complex of "individuality." This is illustrated by Mitchell and Grindley from the records of 18 lots of pigs, each lot consisting of a single litter, ranging from 5 to 10 pigs. In only 3 lots is the probable error greater than the average (11½ per cent.) of all American results as quoted above.

In further illustration of this point the records of a pig-feeding experiment carried out at the Manor Farm, Garforth (Experimental Farm of the University of Leeds), in 1913 are summarised below. A more detailed account of the objects and results of the experiment has been given elsewhere[1]. Ten Large White pigs, about 8 weeks old at the outset, were subjected for 24 weeks (June 30–December 15) to identical conditions of feeding and general treatment. The pigs were drawn from two litters and for convenience were treated as two groups, each drawn from one litter only. With one exception (No. 9) all were castrated males, but owing to the unsatisfactory progress made by No. 7 it was replaced at an early stage of the experiment by a female from the same litter. The data given in the tables for No. 7 refer to this latter animal.

Special feeding pens were used in order to make it possible for each animal to be fed separately and the amount of food supplied was carefully adjusted to the appetite of the least voracious animal. In this way it was ensured that each animal consumed almost exactly the same amount of food. The foodstuffs used were wheat-bran, wheat-"sharps," pea-meal and barley-meal. The pigs were weighed weekly and gave the increases shown in Table I. At the outset difficulties were experienced in getting the pigs accustomed to the separate feeding stalls and to the rations provided. For these reasons the records of the first three weeks are regarded as abnormal and excluded from the table.

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[1] Crowther and Ruston, *Journal Bd. Agric.* xxi. 789.

TABLE I.

	Group A					Group B					Average increase for week	Prob. error of one pig expressed as per cent. of average increase
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10		
						Initial Live-weight (July 21)						
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.	%
	26·5	29·0	29·5	30·5	28·0	30·0	(40·2)*	30·5	32·5	28·0	29·4±·2‡	2⅓‡
						Increase, in lbs.						
4th week	3·0	3·7	2·8	4·6	4·0	3·8	—	3·0	2·6	5·1	3·6±·2‡	16‡
5th "	3·4	3·7	2·5	1·4	2·5	3·0	—	3·7	3·1	1·6	2·8±·2‡	20‡
6th "	4·1	6·0	5·8	7·2	4·1	3·8	—	5·7	4·2	5·0	5·1±·3‡	15‡
7th "	6·5	4·5	7·5	5·6	5·4	5·9	4·8	4·9	4·5	4·0	5·4±·2	13
8th "	4·9	6·5	5·5	10·6	4·5	5·6	5·0	6·4	4·5	7·0	6·0±·4	20
9th "	4·3	6·2	5·0	5·0	6·5	4·6	6·0	4·9	5·1	4·0	5·2±·2	11
10th "	6·9	6·3	8·0	6·7	5·4	6·1	6·0	7·4	5·4	5·9	6·4±·2	9
11th "	8·4	9·5	7·6	8·2	7·5	7·7	8·9	7·8	8·0	6·4	8·0±·2	7
12th "	7·1	10·7	7·5	10·4	7·4	7·0	7·4	8·9	8·2	9·5	8·4±·3	11
13th "	5·5	5·7	5·1	5·9	5·8	5·3	6·7	6·0	6·0	5·3	5·7±·1	6
14th "	7·5	6·0	5·8	5·7	6·1	9·7	6·0	10·6	5·3	8·5	7·1±·4	18
15th "	5·0	4·5	6·5	5·3	3·4	6·0	6·5	2·3	4·8	6·2	5·1±·3	18
16th "	10·3	7·5	6·1	10·7	11·5	8·0	5·5	9·5	6·8	4·0	8·0±·5	21
17th "	11·9	9·5	8·6	5·8	9·4	11·8	13·2	11·5	11·0	13·2	10·6±·5	15
18th "	3·1	6·5	7·2	8·0	1·9	5·3	2·3	5·2	3·5	3·3	4·6±·4	31
19th "	12·1	8·9	7·3	8·2	11·0	9·0	11·3	12·2	8·8	11·1	10·0±·4	12
20th "	4·5	8·2	6·8	8·7	8·8	13·0	10·2	5·8	7·8	9·4	8·3±·5	19
21st "	9·1	5·5	8·0	8·9	8·1	6·8	9·0	11·2	7·1	6·9	8·1±·3	13
22nd "	10·0	12·4	9·2	7·4	12·9	10·5	10·9	8·8	11·7	12·6	10·6±·4	12
23rd "	14·0	13·2	13·3	12·2	11·5	12·0	10·6	9·8	4·1	8·8	11·0±·6	18
24th "	6·5	6·6	3·8	11·5	7·5	11·0	10·0	9·3	11·0	11·7	8·9±·6	20
						Final Live-weight (December 15)						
	174·5	180·8	169·3	188·7	173·0	188·3	181·5	185·2	166·0	177·5	178·5±1·7	3
						Total increase in 21 weeks						
	148·0	151·8	139·8	158·2	145·0	158·3	(141·3)†	154·7	133·5	149·5	148·8±1·9‡	3·7‡

* Weight at end of 6th week (August 11). † Increase for 18 weeks (from August 11).
‡ Average of 9 pigs.

In order to facilitate comparison the data have been condensed in Table II into 3-weekly periods, for which the averages of each group are set out separately.

The results present two features of special interest in the present connection, viz. the extremely low degree of variability between the individual records and the absence of any marked tendency for the probable error (relative to increase) to fall as the feeding progressed.

TABLE II.

Weeks of experiment	Average gain in weight for 3 weeks			Probable error of one pig expressed as percentage of average gain		
	Group A	Group B	All pigs	Group A	Group B	All pigs
	lb.	lb.	lb.	%	%	%
4– 6	11·8± ·4	11·8± ·4*	11·8± ·3†	7·9	7·5*	7·5†
7– 9	17·7± ·7	15·4± ·3	16·6± ·4	8·2	3·8	7·9
10–12	23·5± ·6	22·1± ·4	22·8± ·4	5·6	3·6	5·8
13–15	16·8± ·3	19·0± ·6	17·9± ·4	4·2	6·7	7·9
16–18	23·6± ·4	23·0± ·7	23·3± ·4	3·8	7·2	5·4
19–21	24·8± ·8	27·9± ·8	26·4± ·7	6·7	6·4	8·4
22–24	30·4± ·7	30·6± ·9	30·5± ·6	5·3	6·7	5·7
Average (21 weeks)	148·6±2·1	149·0± 3·7*	148·8± 1·9†	3·1	4·9*	3·7†
Average for 18 weeks (excluding 4th–6th weeks)	136·8±1·8	138·0± 2·6	137·3±1·5	2·9	4·2	3·4

* 4 pigs only. † 9 pigs only.

It will be noted from Table I that, with one exception towards the end of the feeding period, the probable error of one pig never exceeded 21 per cent. of the average weekly increase, whilst for 3-weekly periods (Table II) the maximum (all pigs) was only 8·4 per cent. For the whole period (9 pigs for 21 weeks or 10 pigs for 18 weeks) the probable error of one pig was only about 3½ per cent. of the average increase—as compared with the general average for pigs of 10–12 per cent. quoted by Robinson and Halnan, and by Mitchell and Grindley. If the eight male animals alone be considered the probable error is only 2·9 per cent.

The relative constancy of the probable error throughout the course of the feeding, though not without precedent, is more surprising and difficult to interpret. It may possibly be associated with the system of feeding adopted which ensured equal consumption as contrasted with the equal *chance* of food which ordinary methods of group feeding give to each individual. This may also have contributed to the general low variability in the gains produced.

Obviously the number of animals concerned is so very small that the results cannot be regarded as in any way affecting the validity of the conclusions arrived at from the analysis of more extensive data but they may serve, in conjunction with the American data for separate litters quoted above, to suggest the desirability of a closer study of the possibilities of the pig as an instrument for the measurement of small differences in nutritive value.

(*Received February 6th,* 1915.)

NOTE ON THE EFFECT OF CHANGES IN THE VISCOSITY OF WATER ON THE RESULTS OF MECHANICAL ANALYSES CONDUCTED AT VARYING TEMPERATURES.

By G. W. ROBINSON, B.A.

Adviser in Agricultural Chemistry, University College of North Wales, Bangor.

Some discordant results obtained in mechanical analysis during a spell of cold weather led the writer to enquire into the effect of temperature on the separation of the various fractions of a soil by mechanical analysis.

According to Stokes' law, the limiting velocity of a particle of diameter a falling in a liquid of viscosity η is proportional to $\frac{a^2(\rho - \sigma)}{\eta}$, ρ being the density of the solid, and σ that of the liquid.

Now in any given separation, the depth of liquid and time of subsidence are adjusted so that all particles of diameter greater than a certain value shall be left behind on decantation. If, for any reason, η varies, since v is not changed the diameter, a, of the smallest particles which are left behind on decantation will be proportional to $\sqrt{\eta}$.

The value of η is subject to considerable change when the temperature is varied. For water, Thorpe and Rodger[1] give the formula

$$\eta = \frac{{\cdot}017941}{(1 + {\cdot}023120t)^{1{\cdot}5423}},$$

where t = temperature in degrees centigrade.

The densities, ρ and σ, are also subject to a change with the temperature, but these are of a smaller order of magnitude and may be ignored in comparison with the changes in η.

Calculating $\frac{\eta_{5^\circ}}{\eta_{15^\circ}}$ by this formula we get $\frac{\eta_{5^\circ}}{\eta_{15^\circ}} = 1{\cdot}33$.

That is to say that by a fall of 10° from 15° to 5° C., the viscosity of water increases by 33 per cent. Since the diameter a is proportional to $\sqrt{\eta}$,

$$\frac{a_{15}}{a_5} = \sqrt{\frac{1{\cdot}00}{1{\cdot}33}} = \frac{1{\cdot}00}{1{\cdot}15},$$

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[1] *Phil. Trans.* 1894, A, p. 1.

where a_{15° is the diameter of the smallest particles left behind on decantation at 15° C. and a_{5° the diameter of the smallest particles left behind on decantation at 5° C. From this it is seen that the limiting diameter at 5° C. is 15 per cent. greater than it is at 15° C. If there is a large proportion of particles at or about this limiting diameter it will mean that considerably smaller values will be obtained for the fraction left behind on decantation if the decantation is performed at a lower temperature.

An experiment was performed in which some ignited fine sand was well mixed with an approximately equal quantity of ignited silt. Duplicate two gram lots were taken and after stirring well with water in beakers allowed to settle through 7·5 cm. for 75 seconds. The water was at 6° C. The sedimentations were repeated six times by which time no more silt remained in suspension after 75 seconds. The residual fine sand was collected, dried for several hours and weighed. The fine sand was then mixed again with the silt from which it had been separated at 6° C. and a separation was effected in exactly the same way at 11° C. The fine sand obtained at 11° C. was again dried and weighed. A further separation was made at 16° C. in exactly the same way. The results are as follows.

	Weight of fine sand, grams		
Temperature	*A*	*B*	*Mean*
6° C.	1·053	1·062	1·058
11° C.	1·123	1·114	1·118
16° C.	1·149	1·140	1·144

Putting the weight of fine sand obtained at 6° C. as 100, we have

Temperature	Amount obtained
6° C.	100
11° C.	105·7
16° C.	108·1

This shows that a change in temperature, by altering the viscosity of water, does affect the values obtained in mechanical analysis by sedimentation. It is recommended therefore that sedimentations be carried out, as far as possible, at a uniform temperature, say 12 to 14° centigrade. So far as the writer is able to find out, this point has not up to the present been emphasized.

(*Received March 3rd,* 1915.)

ON THE PROBABLE ERROR OF SAMPLING IN SOIL SURVEYS.

By G. W. ROBINSON, B.A.,
Adviser in Agricultural Chemistry,
AND W. E. LLOYD, B.Sc.,
Research Scholar, University College of North Wales, Bangor.

The following paper records experiments made to determine the magnitude of the error involved in the sampling of a soil for survey purposes. While it is not held that the results obtained are true for all localities, they serve to indicate the order of magnitude of the various errors.

The ordinary method of sampling a soil for survey purposes is, first to select a field uniform in itself and representative of the soil type which is being examined. Borings are then taken in various parts of the field. The borings from the top soil are united to form the soil sample. Similarly those from the subsoil are united to form the subsoil sample. The number of borings recommended in text-books is from five up to ten or twelve according to the size of the field sampled.

Now the probable error affecting the analysis of a single boring is a function of two probable errors, namely, (1) the *laboratory error*, that is the error of analytical determination (which itself includes an error of sampling from the laboratory sample), and (2) the *field error*, which is the error due to the normal variation in the composition of the soil from point to point in the field.

Now if the probable error of a determination on one boring be P, the probable laboratory error be p_1, and the probable field error be p_2,

$$P = f(p_1 p_2).$$

P, p_1 and p_2 will have different values according to the soil constituent which is determined. There will thus be a different set of P, p_1 and p_2

for nitrogen, phosphoric acid, coarse sand and so on. By a well-known formula

$$P = \sqrt{p_2^2 + p_1^2},$$

provided that, as may be assumed to be the case, the errors are independent.

The object of this investigation is to obtain values of p_2, the probable field error, for various soil constituents.

There is practically no literature on the subject. The only account of previous work accessible to the writers is in a paper by J. W. Leather[1]. Leather took duplicate samples from several plots in different localities. Each sample consisted of a mixture of a dozen borings. He found that the differences between determinations on the duplicates of nitrogen, available phosphoric acid and potash respectively, varied from nothing up to more than 20 per cent. Unfortunately the data do not give any basis for determination of probable errors due to field variation[2].

There are various papers which treat of the probable error of experimental plot yields, but since the composition of the soil is only one factor in determining crop yields, it cannot be expected that these probable errors are any measure of the probable error of sampling.

Two fields were therefore investigated, one on a drift and the other on a sedentary soil. The following notes describe the two fields.

Field A, at the College Farm, Aber, near Bangor. This field has been for many years in grass. The soil is glacial drift of local origin. It is not particularly uniform in texture and appearance and for ordinary survey purposes would be reckoned too variable.

Field B, at Cellar, Aberffraw, Anglesey. This field is now in arable cultivation. The soil is derived from the Pre-Cambrian schists. There is a certain admixture of wind blown sand since the field is not far from the sea. The soil is quite uniform in texture and appearance.

In Field A, 25 samples were taken. The diagram, Fig. 1, shows the order in which the borings were made.

Each sample was kept separately in a bag. On arrival at the laboratory all the samples were analysed according to the usual methods. The following analyses were performed: Mechanical analysis, determination of hygroscopic moisture and organic matter, and the determination of the total P_2O_5. In the case of the P_2O_5 determinations it was felt that the error of analysis would be smaller if absolute determinations were made. Accordingly, the total P_2O_5 was determined by treatment with sodium peroxide.

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[1] *Trans. Chem. Soc.* 1902, p. 883.

[2] Since sending in this paper, the authors have seen a paper by Pfeiffer and Blanck, *Landw. Versuchs.-Stat.* LXXVIII. Working on the nitrogen content of an experimental field, they obtained a field error of 2·5 per cent. This was, however, over a small area and the result is scarcely of service for survey purposes.

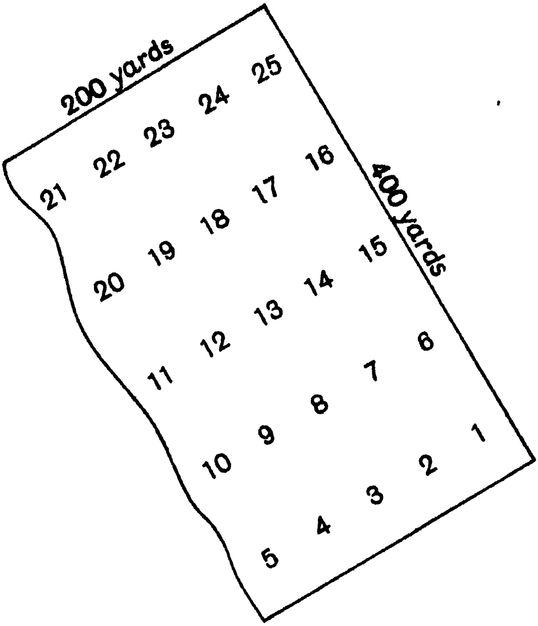

Fig. 1. Field A.

In order to get some idea of the reliability of the figures obtained, *i.e.* to determine p_1, six mechanical analyses and P_2O_5 determinations were made of a well mixed composite sample of a number of borings. The results are shown in the following table.

Composite Sample.

	Fine gravel	Coarse sand	Fine sand	Silt	Fine silt	Clay	P_2O_5
1	7·93	16·31	15·42	15·30	24·21	4·80	·343
2	7·79	18·24	15·75	14·84	23·81	5·06	·310
3	7·90	17·56	15·25	14·73	24·65	4·20	·331
4	9·67	17·78	15·53	13·65	22·80	4·88	·315
5	8·03	17·75	15·71	14·45	24·44	5·09	·323
6	8·02	18·17	14·99	14·42	24·96	4·87	·315

The mean values and probable errors calculated from these figures are as follows:

Constituent	Mean value	Probable error
Fine gravel	8·22	± ·52 = ± 6·3 per cent.
Coarse sand	17·63	± ·54 = ± 3·0 ,,
Fine sand	15·43	± ·20 = ± 1·3 ,,
Silt	14·56	± ·38 = ± 2·6 ,,
Fine silt	24·14	± ·52 = ± 2·1 ,,
Clay	4·81	± ·11 = ± 2·3 ,,
P_2O_5	·323	± ·008 = ± 2·5 ,,

Similar determinations were not made for moisture and organic matter. The determinations are made with much less trouble than the mechanical analyses and P_2O_5 estimations, and therefore the moisture and organic matter were determined four times on each boring and averaged. The agreement was so close in all cases that p_1 for these determinations would be exceedingly small both absolutely and in comparison with the corresponding values of P determined.

Fine gravel	Coarse sand	Fine sand	Silt	Fine silt	Clay	Moisture	Organic matter	Phosphorus pentoxide (P_2O_5)
2·43	14·56	18·33	13·78	27·63	4·18	3·65	12·63	·304
7·33	14·80	19·37	11·79	26·47	4·11	4·03	9·94	·261
7·84	16·02	19 20	10 37	27·99	4·06	3·24	8·55	·279
7·34	17·27	21·35	8·59	26·73	4·07	3·77	8 59	·256
7·60	15·95	16·89	10·10	29·38	5·28	3·21	8·89	·261
11·21	20·32	18 09	8·60	22·65	3·46	2·99	10·91	·366
5·32	18·71	16·60	14·95	24·30	3·66	3·62	11·33	·312
17·25	20·36	14·46	11·31	20·10	3·26	2·41	9·71	·329
12·14	19·57	14·91	12·02	21·93	3·00	2·84	12·25	·318
8·37	17·07	19·59	11·45	24·90	3·64	3·13	10·06	·281
7·42	14·59	20·87	13·56	25·12	4·47	2·46	9·61	·314
2·63	11·33	23·37	14·12	28·03	5·54	2·32	9·59	·286
12·49	22·20	14·35	11·51	21·45	3·42	2·07	9·22	·295
11·20	16·16	14·72	13·84	24·92	3·54	2·26	11·26	·298
7·72	16·20	15·67	13·41	27·34	4·35	1·92	10·30	·214
2·83	11·78	15·31	16·13	33·52	5·40	2·10	10·30	·229
8·20	17·90	21·38	12·40	24·77	4·30	1·63	10·75	·250
15·35	19·92	16·09	10·40	21·44	3·79	2·30	9·10	·198
10·56	22·52	15·52	11·39	21·25	4·01	2·63	9·65	·254
7·38	21·48	15·13	12·93	20·00	3·40	2·79	12·72	·440
11·49	20·79	15·74	10·57	22·65	4·90	2·07	9·81	·317
4·73	22·25	15·96	13·43	21·12	3·75	2·96	13·14	·435
4·00	19·25	16·76	15·28	24·25	3·58	2·68	11·69	·303
2·22	11·92	19·83	15·96	31·53	4·01	2·53	10·16	·267
3·10	16·96	19·66	13·21	26·50	6·25	2·40	8·89	·198

It will be seen from the values obtained above that with the exception of the fine gravel p_1 is about 2·5 per cent. as a maximum[1]. These figures are only obtained from six sets of determinations, but they are sufficiently accurate for the purpose of determining p_2, as will be seen later.

The table on p. 147 shows the results of the analyses of the 25 samples from Field A.

The mean values and probable errors calculated from these results are as follows:

	Per cent.	Probable error per cent. of result, P
Fine gravel	7·92 ± 2·78	= ± 35·0 per cent
Coarse sand	17·59 ± 2·24	= ± 12·7 ”
Fine sand	17·61 ± 1·64	= ± 9·3 ”
Silt	12·44 ± 1·24	= ± 10·0 ”
Fine silt	26·50 ± 2·71	= ± 10·2 ”
Clay	4·10 ± ·52	= ± 12·7 ”
Moisture	2·7 ± ·40	= ± 14·8 ”
Organic matter	10·4 ± ·80	= ± 7·7 ”
Phosphorus pentoxide (P_2O_5)	·290 ± ·030	= ± 13·4 ”

In Field B a set of 15 borings was taken as shown on the diagram Fig. 2.

The following results were obtained on analysis:

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Fine Gravel	Coarse sand	Fine sand	Silt	Fine silt	Clay	Moisture	Organic matter	P_2O_5
4·45	43·39	18·04	8·52	12·91	3·45	2·16	5·54	·220
5·73	44·12	16·57	8·42	12·83	3·20	2·15	5·32	·209
6·13	40·79	16·23	8·81	13·95	3·15	2·42	6·10	·249
4·15	40·41	16·80	8·55	14·55	3·50	1·33	6·78	·214
7·57	42·30	16·44	8·78	14·15	3·20	1·46	5·53	·220
5·58	42·05	16·59	9·20	14·15	3·01	2·14	5·21	·204
6·05	38·97	18·37	10·06	14·86	3·64	2·09	5·22	·188
5·24	47·50	14·39	8·70	13·60	3·14	2·02	5·30	·199
3·37	46·74	16·23	9·53	12·85	3·30	2·00	5·26	·223
4·50	47·36	16·02	8·58	12·34	3·25	1·97	4·74	·264
4·95	43·56	17·11	8·98	12·86	3·82	1·94	5·23	·217
6·64	40·85	17·00	9·65	14·16	3·36	2·00	4·76	·179
4·77	43·43	16·98	8·12	13·01	3·36	2·13	5·17	·269
4·02	46·25	16·42	7·53	12·82	3·02	1·75	5·25	·229
5·40	47·46	16·56	7·35	11·49	3·17	2·06	4·99	·213

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[1] In mechanical analyses this error will be much greater if the temperature of sedimentation is not constant, owing to viscosity changes.

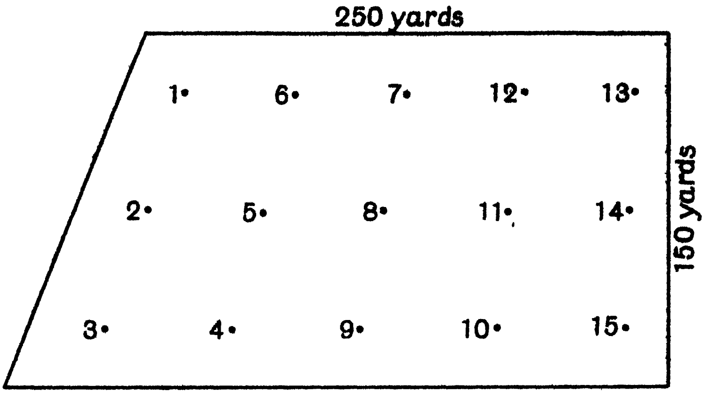

Fig. 2. Field B.

The mean values and probable errors calculated from these results are as follows:

	Per cent.	Probable error per cent. of result, P
Fine gravel	5·23 ± ·73	±13·9 per cent.
Coarse sand	43·68 ± 1·76	± 4·0 „
Fine sand	16·65 ± ·45	± 2·7 „
Silt	8·73 ± ·48	± 5·5 „
Fine silt	13·37 ± ·60	± 4·5 „
Clay	3·30 ± ·13	± 3·9 „
Moisture	1·97 ± ·18	± 9·2 „
Organic matter	5·36 ± ·235	± 4·4 „
Phosphorus pentoxide (P_2O_5)	·220 ± ·017	± 7·7 „

The results obtained may now be stated:

Field A. Drift Soil.

Determination made	P (per cent.)	p_1 (per cent.)	$p_2\ (=\sqrt{P^2-p_1^2})$ (per cent.)
Fine gravel	35·0	6·3	34·5
Coarse sand	12·7	3·0	12·3
Fine sand	9·3	1·3	9·2
Silt	10·0	2·6	9·7
Fine silt	10·2	2·1	10·0
Clay	12·7	2·3	12·4
Moisture	14·8	v. small since mean of 4 was taken	14·8
Organic matter	7·7		7·7
P_2O_5	13·4	2·5	13·2

Field B. Sedentary Soil.

Determination made	P (per cent.)	p_1 (per cent.)	$p_2 \left(=\sqrt{P^2 - p_1^2}\right)$ (per cent.)
Fine gravel	13·9	6·3	12·4
Coarse sand	4·0	3·0	2·6
Fine sand	2·7	1·3	2·4
Silt	5·5	2·6	4·8
Fine silt	4·5	2·1	4·0
Clay	3·9	2·3	3·1
Moisture	9·2	v. small since mean of 4 was taken	9·2
Organic matter	4·4		4·4
P_2O_5	7·7	2·5	7·3

Practically the same values for p_2 might have been obtained if analyses had been repeated several times for each boring and averaged. The values of p_1 would then have been greatly reduced and consequently, as in the case of the moisture and organic matter, p_2 could be taken as approximately equal to P.

In view of the great time taken in making soil analyses it was felt that the increase in the accuracy of the values of p_2 which would thus be obtained would scarcely be great enough to justify spending four or five times as long over the laboratory work. Also, no time was spent in doing chemical analyses other than the determination of P_2O_5.

It is seen from the above tables that in the case of Field A, which is too variable for ordinary survey purposes, p_2 is as great as 31·5 per cent. for fine gravel. Now this constituent is comparatively unimportant in characterising a soil unless it predominates over other fractions. An accurate knowledge of the hygroscopic moisture is also, for survey purposes, of no great importance. Of the other determinations the probable field errors of the fractions in the mechanical analysis are about 10 per cent., while that of the P_2O_5 is 13·4 per cent.

If therefore we count on a probable error of 13·4 per cent. for this soil this can be taken as a maximum value. Similarly 7·3 per cent. can be taken as the maximum value of the field error for Field B which is a sedentary soil. Field A is as stated above of too variable a character for ordinary survey purposes. If therefore operations are conducted on the basis of a maximum field error of 10 per cent. it will probably serve for survey work.

The value of the total probable error of the final result can now be reduced to any desired value by increasing the number of borings and the number of determinations.

Thus if n borings are made and one determination made on each boring the probable error of the average is

$$\pm\frac{\sqrt{p_1^2+p_2^2}}{\sqrt{n}}\text{ or }\frac{P}{\sqrt{n}}.$$

For example, in the case of P_2O_5 in Field A, 9 borings separately analysed and averaged would give a probable error of

$$\pm\frac{13\cdot4}{\sqrt{9}}=\pm\,4\cdot46\text{ per cent.}$$

If n borings are made and a composite sample obtained by mixing, the field error will be reduced to $\frac{p_2}{\sqrt{n}}$, but the laboratory error will still be present, so that if 9 borings from Field B were mixed, the error of one analysis would be

$$\pm\sqrt{2\cdot5^2+\left(\frac{13\cdot2}{\sqrt{9}}\right)^2},\text{ or }\pm\,5\cdot05\text{ per cent.}$$

Where the field error is large in comparison with the laboratory error not much additional accuracy is obtained by analysing the borings separately. Also, however many borings are taken and mixed, it is not possible to reduce the final error to less than the laboratory error; and it is also somewhat difficult to ensure the satisfactory mixing of a very large number of borings.

Performing more than one analysis on a composite sample will serve to reduce the laboratory error, and consequently the final error of result.

If the field error for chemical analyses be taken as ± 10 per cent. and that for the mechanical analyses as ± 5 per cent., we shall probably not err on the side of minimising errors, since no field is sampled for survey purposes unless it appears fairly uniform. As is mentioned above, Field A would not be considered uniform enough for survey purposes.

The following table shows the probable error of final result for different numbers of borings and for single and duplicate analyses of the mixed sample. The probable errors are calculated on the assumption that the final probable error is equal to the square root of the sum of the squares of the separate probable errors. Thus the probable error of an average of two determinations on a composite sample of 5 borings is in the case of mechanical analyses equal to

$$\pm\sqrt{\left(\frac{2\cdot5}{\sqrt{2}}\right)^2+\left(\frac{5}{\sqrt{5}}\right)^2}=\pm\,2\cdot8\text{ per cent.,}$$

and in the case of a chemical analysis,

$$\pm\sqrt{\left(\frac{2\cdot5}{\sqrt{2}}\right)^2+\left(\frac{10}{\sqrt{5}}\right)^2}=\pm\,4\cdot8 \text{ per cent.}$$

Number of borings taken and mixed to form composite sample	Final probable error, per cent.			
	Mechanical analysis ($p_2 = \pm$ 5 per cent.)		Chemical analysis ($p_2 = \pm$ 10 per cent.)	
	Single analysis	Average of two analyses	Single analysis	Average of two analyses
1	±5·6	±5·3	±10·3	±10·1 %
2	±4·3	±3·95	± 7·5	± 7·3
3	±3·8	±3·5	± 6·3	± 6·0
4	±3·5	±3·05	± 5·6	± 5·3
5	±3·3	±2·8	± 5·1	± 4·8
6	±3·2	±2·7	± 4·8	± 4·4
7	±3·1	±2·6	± 4·5	± 4·2
8	±3·05	±2·5	± 4·3	± 3·95
9	±3·0	±2·4	± 4·15	± 3·75
10	±2·95	±2·35	± 4·0	± 3·6

The table shows that even with 10 borings there is not much diminution in the probable error by taking an average of two analyses. Since however some check is advisable it is better to make two analyses.

When two mechanical analyses are performed it is seen that not much additional accuracy is obtained by increasing the number of borings beyond six, either in the mechanical or chemical analyses. Taking six as the number of borings the probable error is ± 2·7 per cent. This means that it is an even chance that a result obtained is within 2·7 per cent. of the true result. The chances of a result having lower degrees of accuracy are shown in the following table, calculated from Wood and Stratton's paper on the Interpretation of Experimental results[1].

Percentage deviation from true value	Chances of result being within limits of such deviation
2·7 per cent.	Evens
3·4 ,,	3 to 2
3·9 ,,	2 to 1
4·6 ,,	3 to 1
5·1 ,,	4 to 1
5·4 ,,	9 to 2

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[1] This *Journal*, Vol. III. p. 429.

Similarly in the case of a chemical analysis, we have for six borings:

Percentage deviation from true value	Chances of result being within limits of such deviation
4·4 per cent	Evens
5·5 ,,	3 to 2
6·3 ,,	2 to 1
7·5 ,,	3 to 1
8·3 ,,	4 to 1
8·8 ,,	9 to 2

From these results it is seen that an accuracy of 5 per cent. in mechanical analysis is ensured a probability of 4 to 1 by doing a duplicate analysis on six borings. For survey purposes this is probably sufficient, since it is not conceivable that variation in the amount of any fraction corresponding to 5 per cent. (relative to the amount of the fraction) could have any effect on the properties of a soil.

In the case of chemical analysis it does not seem that the same accuracy can be expected. For survey purposes however the accuracy is probably sufficient. In the case of a critical study of one soil however it would be necessary to reduce the errors much more by repeating analyses and increasing the number of borings.

(*Received March 3rd,* 1915.)

THE STARCH EQUIVALENT THEORY.

By J. ALAN MURRAY, B.Sc.

(*University College, Reading.*)

The account given by Wood and Yule of their investigation of the records of British Feeding Trials and the Starch Equivalent Theory in part 2 vol. VI of this *Journal* is a valuable and timely paper. It contains abundant material for reflection. Consideration of the data suggests that there is room for doubt in regard to the conclusions for which a claim to "certainty" has been advanced. It is not, however, the immediate purpose of this article to discuss these conclusions—though incidentally certain points in the investigation come under review—but rather to discern, as far as possible, the direction in which the methods and arguments employed lead, and more particularly what is their bearing on the starch equivalent theory as a practical system.

In their opening remarks Wood and Yule state that "starch equivalent for this purpose (maintenance) is reckoned by the formula: starch equivalent = digestible protein × 1·25 + digestible fat × 2·3 + amides × 0·6 + digestible carbohydrates + digestible fibre. The generally accepted maintenance ration for a 1000 lb. ox on this basis is 6·35 lb. of starch equivalent which includes 0·6 lb. of digestible protein." The author is not concerned to deny these statements but he desires to emphasise the fact that this formula is not Kellner's formula and that this estimate of the animal's requirements is not Kellner's estimate.

The formula differs from that of Kellner[1] in two respects. In the latter the protein is multiplied by 0·94 instead of 1·25, and a further correction which varies according to the estimated percentage value for fattening of each food is also made. The formula given by Wood and Yule is, as stated, intended to determine the value of the foods for maintenance. Hence these differences. But, it may be asked, is it seriously proposed that the starch equivalent for maintenance should be reckoned by one formula and that for fattening by another? If so, the starch equivalent system will scarcely survive the shock, for it would be equally necessary to have another formula for milk production and still another for work. Kellner's system is fundamentally a mere convention. It does not establish a true "quantitative relationship between the amount of food and the amount of fat, work or milk it may be expected to produce." Its general applicability to these problems rests essentially upon uniformity of the method, and if this is undermined its more important advantages are destroyed.

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[1] *The Scientific Feeding of Animals*, p. 355

The factor 1·25 is presumably based on the relative amounts of heat evolved from protein and starch when these substances are oxidised in the animal's body, whereas the factor 0·94 is based upon the relative amounts of fat formed from them. This point appears to have been appreciated by Kellner and to have been allowed for by him as shown below.

It is generally agreed that the total thermic energy of the digestible matter of the food is available for maintenance. It is, apparently, for this reason that Wood and Yule make no correction for the "value" of the food. Such correction is, however, the very essence of Kellner's system, and if it be not made the numbers should not be called "starch equivalents." The formula given by Wood and Yule is a reversion to the system of Wolff and his colleagues and predecessors. It differs from what was formerly called "total digestible nutrients" only in respect of the factor (1·25 instead of 1·0) applied to the protein; and so far as the foods commonly used for maintenance rations are concerned this difference is insignificant. The author suggests that if such reversion is to be countenanced it would be well to revert to the old-fashioned or some other name. Endless confusion must result if the term "starch equivalent" be promiscuously applied to essentially different things.

This at once becomes evident when we turn to consideration of the requirements of the animals. The specious similarity between Kellner's[1] estimate of 6 lb. and that of Wood and Yule of 6·35 lb. of starch equivalent per day for maintenance of a 1000 lb. ox is deceptive. The latter, according to the formula given, includes the whole of the thermic energy of the digestible matter of the food; the former corresponds to the dynamic portion only. In order to make this quite plain Kellner

[1] *The Scientific Feeding of Animals*, p. 392.

shows in his appendix tables[1] that the maintenance ration from which his 6 lb. of starch equivalent is derived must contain 0·6 to 0·8 lb. of digestible protein, 0·1 of digestible fat and 7·5 to 9·5 lb. of digestible carbohydrates and fibre. This amounts to from 8½ to 10¾ lb. of "starch equivalent" reckoned by the formula given by Wood and Yule.

We have here to deal with a serious defect in Kellner's system. He found[2] that, on the average, only some 50 to 70 per cent. of the available energy of the foods used for maintenance rations is of dynamic value. Consequently the starch equivalents of these foods are from 30 to 50 per cent. below the full value. The maintenance requirements of the animals, when expressed in terms of starch equivalent, must therefore be reduced by a similar amount. The quantities of feeding stuffs necessary to maintain the animals may be estimated with approximate accuracy on this basis provided the foods are of the value assumed in the estimate of the animal's requirements, but not otherwise. For example, the quantity of maize meal which corresponds to 6 lb. of starch equivalent would be too small for maintenance of a 1000 lb. ox because it yields little or no thermic energy in addition to that represented by the starch equivalent In practice, of course, no one would use maize meal for maintenance rations of oxen; but in the investigation undertaken by Wood and Yule the fattening ration (most of which it may be assumed was of nearly full value) was assigned for maintenance functions in order that the fattening value of the roots might be determined. In this case 6 lb. of Kellner's starch equivalent, derived from such foods, would be inadequate. As Wood and Yule did not employ Kellner's formula but another which includes the whole of the available energy of the food, this argument does not apply. It remains to be seen, however, whether the 6·35 lb. of starch equivalent, reckoned by that formula, was sufficient.

It will be seen that Kellner's estimate of 6 lb. of starch equivalent for maintenance is a purely artificial number arbitrarily adapted to the needs of his convention. That being so, it makes little difference whether the protein be multiplied by 1·25 or by 0·94. The net result of using the higher factor is that the estimates of the requirements for maintenance must be correspondingly increased. Kellner appears to have deliberately decided to ignore the difference between the heat producing and fat producing values of protein in order to attain that uniformity which his system demands, and because he saw that it could easily be allowed for in his empirical estimate of the animal's requirements for maintenance.

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[1] *The Scientific Feeding of Animals*, p. 392.

[2] *Ibid.*, p 360 *et seq.*

Six pounds of Kellner's starch equivalent corresponds to about 20 lb. of hay of average quality. This estimate of the requirements for maintenance of a 1000 lb. ox does not differ materially from that of Wolff[1] and the older authorities, and the author has hitherto regarded it as "the generally accepted ration." It is, however, very different from that of Wood and Yule, which corresponds to only about 13 lb. of such hay. This discrepancy cannot be lightly passed over. It is too considerable, and it is of fundamental importance in regard to this and other investigations. If they adhere to their statement it would be interesting to know upon what data it is founded. It is to be hoped that they will take an early opportunity of clearing up the matter.

It is of course possible that Wood and Yule did not intend to propose a standard radically different from that of Kellner and Wolff. The omission from their formula of all reference to the "value" of the food may be merely a typographical error, but the statement that an ox of 1000 lb. live weight when on maintenance diet evolves 10,800 Cal. of heat per day seems to preclude this explanation. It is evident that this statement is not based upon a direct determination in the calorimeter, but that 10,800 Cal. is merely the calculated equivalent of 6·35 lb. of pure starch. It may be inferred therefore, that the formula was intended to include the whole of the available energy of the food and not the dynamic portion only. The only obvious way of escape from this conclusion is to assume that the statement involves a repetition of Kellner's mistake in calculating the amount of heat an animal should evolve from the starch equivalent instead of from the total available energy of the food. He says[2] that "an ox weighing 600 Kg. requires a daily supply of 12,780 Cal."; but as this corresponds to about 10,620 Cal. for an ox of 1000 lb. it is clearly inconsistent with the ration prescribed in the tables. The latter, quoted above, represents from 14,500 to 18,350 Cal. per day of available energy, and this accords well with the estimate on the next page: "For maintenance of the animal determined from other experiments- 17,320 Cal."

It has hitherto been customary to assume that the requirements for maintenance of oxen were proportional to the live weights of the animals and could therefore be determined by rule of three, though it has long been known that this is not true. A notable advance has been made by Wood and Yule in discarding this assumption. They determined the variation in the requirements of oxen of different sizes by means of a diagram, which by their kind permission is reproduced below

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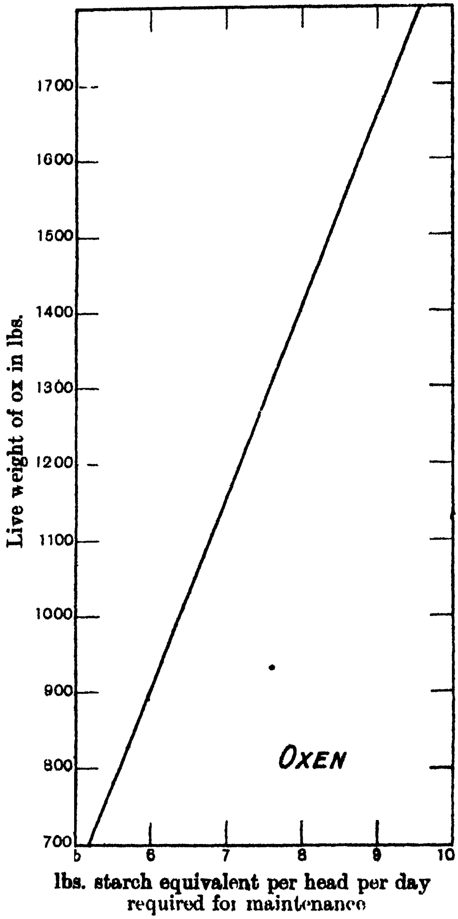
[1] *Farm Foods* (p. 350). Gurney and Jackson, 1895.

[2] *The Scientific Feeding of Animals*, p. 48.



Reduced to numerical terms, this graph may be expressed by the formula:

$$\log E = \tfrac{2}{3} \log M - 1{\cdot}19723,$$

M is the live weight (lb.) of the animal and *E* is the amount (lb.) of starch equivalent required for maintenance.

The concordance between the graph and the formula is shown below:

M	800	1000	1200	1400	1600	1800
E (graph)	5·5	6·35	7·2	8·0	8·8	9·4
E (formula)	5·47	6·35	7·17	7·95	8·69	9·4

The figures from which the graph was constructed were taken from Kellner's Ernährung. They are not the results of direct determinations of food requirements, but, if the author is not mistaken, are derived from measurements of the relation between the body surface and live weight of certain dogs; and they involve the assumption that the requirements for maintenance vary directly as the extent of body surface. It is well that this should be recognised, but there is good reason to anticipate that when it is put to the test of experiment the assumption will prove to be well founded. Over a year ago the author published a formula[1] for maintenance rations of oxen in terms of "total digestible nutrients with an albuminoid ratio of 10 – 1." Assuming that 9·3 lb. of such nutrients is the maintenance ration for oxen of 1000 lb. live weight, this formula may be expressed as follows:

$$\log N = \tfrac{2}{3} \log M - 1{\cdot}03152.$$

This view is confirmed by independent data of a purely mathematical kind. It also derives a certain amount of support from the fact that the requirements of animals of 40 to 170 lb. live weight, calculated by this formula, are consistent with experimental data relating to the maintenance rations of pigs of that size[2]. The argument cannot be applied to the formula in terms of starch equivalent because the foods that are suitable for maintenance of pigs are of nearly "full value," *i.e.* they contain no thermic energy over and above the dynamic portion represented by the starch equivalent. It might be supposed that it could be applied in the case of other ruminants such as sheep, which can subsist on much the same kind of diet as oxen. Such, however, is not the case, because, owing to their thick coats of wool, these animals do not lose heat by radiation so rapidly as pigs and oxen in proportion to their body surface.

Wood and Yule have assessed the maintenance ration for sheep of 100 lb. live weight at about 6½ lb. of starch equivalent per week; but, on the ground that this shows a higher percentage utilisation of the food for fattening, they suspect that the allowance is too small. If the starch equivalent referred to was calculated by the formula given by them, *i.e.* if it includes the whole of the available energy of the food, there can be little doubt that it is too small. On the other hand, if it means Kellner's starch equivalent, it is probably too large.

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[1] *The Chemistry of Cattle Feeding and Dairying* (Longmans), pp. 128 and 142.

[2] Sanborn's *Expers.* Bul. 28, Mo. Agr. Col. Cf. *Chemistry of Cattle Feeding and Dairying*, p. 206.

The author's estimate[1] of the maintenance requirements of sheep of 100 lb. live weight is from 7½ to 8¼ lb. per week of "total digestible nutrients" corresponding to about 5 or 5½ lb. of Kellner's starch equivalent. Even so, there is reason to believe that sheep retain in their bodies a larger percentage of the food in excess of the maintenance ration than oxen do.

The whole question stands in urgent need of re-examination on a fundamental basis. The estimate that the fattening increase comprises "67 per cent. of dry matter, chiefly fat, and 33 per cent. of water" is probably true only under conditions similar to those of the Rothamsted experiments in which the animals[2] appear to have been not fully grown. Comparison of the figures[3] relating to the fat ox and the half-fat ox shows that of a fattening increase of 200 lb. something like 190 lb. was fat and only 10 lb., *i.e.* about 5 per cent., was water. It may ultimately prove that the true fattening increase apart from growth -consists almost entirely of pure fat.

It is to be noticed also in this connection that in the attempts to estimate the maintenance rations of oxen of different size by means of graphs or formulae it is assumed that all the animals are in store condition, *i.e.* that the difference in live weight is due in all cases to greater or less growth and not to fattening. Fat animals require more food to maintain them without gain or loss of weight than those in lean condition. It is not easy to see how a single graph could be adapted to meet this difficulty, but the formula given above might be modified in some such manner as that shown below:

$$\log N - \tfrac{2}{3} \log M - 1{\cdot}03152x,$$

where x is a number that varies inversely as the fatness of the animal. If any means can be found to express the degree of fatness in numerical terms it would probably not be very difficult to determine x. All that can be said at present is that when the animals are in store condition – whatever that may be –the value of x is 1, and that when they are fatter it is less than 1.

It may also be found eventually that herein lies the explanation of the gradually diminishing returns in the shape of increase for food consumed as the animal becomes fatter. To say that it is due to the fact that a fat animal requires more food for maintenance is not merely a repetition of the statement in different terms, because, if true, it would be equally applicable to an animal producing milk.

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[1] *Chemistry of Cattle Feeding and Dairying* (Longmans), pp 130 and 204.

[2] Cf *Scientific Feeding of Animals*, pp 254 and 255

[3] *Rothamsted Memoirs*, Vol III. pp 520 and 558 Cf *Chemistry of Cattle Feeding*, pp 189 and 190

The starch equivalent system is still on trial, and it is tolerably evident that it is far from perfect. It is complex in definition and difficult to apply. Farmers as a rule will have none of it, and it frequently proves a stumbling-block to scientific students. It may even betray experts as it has on occasion betrayed Kellner himself. The system originated in a praiseworthy attempt to overcome some of the more obvious objections to the old-fashioned "feeding standards" in which it was assumed that the nutrients in all kinds of foods were of equal value for all kinds of purposes. At least it is in that direction that it has found its chief application. The foregoing discussion, however, tends to show that the day of such feeding standards is over. Attempts to calculate a ration comprising two or more independent variables, *e.g.* maintenance and fattening, by a single arithmetical operation – the rule of three which it now appears is not applicable to either are no longer defensible. Conversion of the nutrients into starch equivalents does not overcome this difficulty.

Animals require food for maintenance and for the several forms of production—growth, work, fattening, and lactation –though it rarely happens in practice that more than two, or at most three, out of these five conditions have to be satisfied at the same time in any given case. The best results will, therefore, be obtained when the digestible nitrogenous and non-nitrogenous nutrients are supplied in the proportions and quantities required for each specific purpose. The amounts required for maintenance depend upon the size of the animal and those for other purposes upon the rate of each kind of production, though probably in no case are they directly proportional to that rate. In each case the nutrients must be derived from a food suitable for the purpose. For example, the nutrients for maintenance of oxen should be derived from cheap coarse fodders, and those for fattening from the finest, most readily digestible materials. Nothing should be deducted from the former for the work of digestion, etc. From the latter there is nothing to deduct on this account. At least, except in one or two instances, the amount to be deducted is insignificant. If, however, the amounts of nutrients for the several purposes are not to be added together but directly translated into the corresponding amounts of appropriate kinds of food, it seems clear that they must be determined by a separate calculation in each case, and the *raison d'être* of the starch equivalent system disappears.

Elsewhere[1] the author has proposed that the feeding standards should be superseded by formulae. These should be in terms of "total digestible nutrients" with given albuminoid ratios for maintenance, growth, work, fattening and milk production. This would afford a simple and satisfactory solution of difficulties hitherto encountered, and it possesses many collateral advantages. Perhaps not the least of these is the fact that attempts to evolve such formulae would effectually expose our ignorance of many points at present concealed by the feeding standards and further obscured by the complexities of the starch equivalent system. In the present state of our knowledge indeed such formulae could be little more than hypotheses but they would serve to give point and direction to research.

Wood and Yule's paper shows that some progress has already been made in this direction and it encourages the hope that in the near future the rate of advance may be greatly accelerated.

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[1] *Chemistry of Cattle Feeding and Dairying* (Longmans)

(*Received January 1st,* 1915.)

THE MAINTENANCE RATION OF OXEN AND THE STARCH EQUIVALENT THEORY.

BY E. T. HALNAN, M.A.

(Animal Nutrition Institute, Cambridge.)

WITHIN recent years, Kellner's system of starch equivalents has received much criticism from various quarters, and its value as a practical feeding system has been called into question. That the system is far from perfect in its working is universally admitted, and the recent work of W. Bruce[1] with oxen has done much to emphasise this fact. In a series of practical feeding trials with oxen, Bruce obtained results contrary to those expected from the theoretical consideration of the Kellner system of starch equivalents, and largely on account of this work, and partly on account of the demonstration of the importance of the part played by certain unknown chemical compounds in the normal metabolism of animals, many workers have been led to suggest a total abolition of the Kellner system. It is, however, inconceivable to the author that a system based on 30 years continuous research upon feeding problems, should be condemned without the fullest possible enquiry. Furthermore it is supported by many experimental results. In a critical comparison of the values of certain foods for milk production theoretically computed by the Kellner system, Crowther[2] has shown that the results obtained with one exception agree very well with those obtained in actual feeding practice. Moreover, with regard to the food deficiency factors which play such an important role in the normal metabolism of animals, Hopkins[3] has shown that those factors are hardly likely to be missing in normal dietaries, and consequently the results obtained in ordinary feeding trials will rarely be affected by lack of these factors.

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[1] Bruce "Cattle Feeding Experiments," *Edinburgh and E. of Scot. Coll of Ag. Report*, **27**.

[2] Crowther, *Science Progress*, **7**, p. 436

[3] Hopkins, *Journ. of Phys* 1912, **44**, p. 425.

Two papers of importance on the subject of starch equivalents have recently appeared, that of Wood and Yule[1], in which an attempt has been made to explain the discrepancies existing between the German figures and the results obtained in English feeding practice; and the more recent paper of Murray[2], which not only favours total abolition of the Kellner system, but also criticises the accuracy of the results stated by Wood and Yule. The present paper has, therefore, been written with a view to clearing up the points dealt with by Murray in his paper, and criticism of Murray's views has consequently been confined to those points which bear directly on the subject under discussion.

In the first portion of the paper, Wood and Yule are criticised for suggesting the necessity of distinguishing between "starch equivalent for maintenance" and "starch equivalent for production." Recognising the difference between the thermic and dynamic values of foods, and the fact that within reasonable limits the total digestible thermic energy of a food is available for maintenance, Wood and Yule give two different formulae to be applied according as the food is intended for maintenance purposes or fattening purposes. In doing so they thus render easy a distinction between the thermic and dynamic value of a food, and one of Murray's most serious objections to the Kellner system disappears. It may be as well here to define clearly what Kellner intended by his system. Kellner aimed at giving a measure of the available energy of the excess of any ration over the maintenance ration by measuring the amount of fat produced compared with the amount of fat produced by an equal quantity of starch. The available energy once determined and stated as starch equivalent may be readily calculated to work production, milk production, or any other purpose. The starch equivalents so determined were intended to be used solely for estimating the relative fattening capacities of various foods, and were not intended to be applied indiscriminately for the estimation of maintenance and also for the production of fat. The endeavour to do so naturally led to much confusion of thought as to the exact meaning of "starch equivalent," and the attempt to interpret maintenance requirement in terms of "starch equivalent," *i.e.* "starch equivalent for fat production," caused even Kellner to confuse the issue. Under these circumstances, it is difficult to understand Murray's objection to Wood and Yule's use of "maintenance starch equivalent," especially since Murray himself admits that "much confusion of thought must result if the term 'starch equivalent' be promiscuously applied to essentially different things" and also clearly shows the impossibility of completely defining starch equivalent for maintenance by starch equivalent for production alone.

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[1] Wood and Yule, *Journ. of Agric. Sci.* **6**, p. 233.

[2] Murray, *ibid.* **7**, p. 154.

The term "starch equivalent" in the sense used by Kellner is a scientifically incomplete term, since it does not express in itself all it is intended to express, but may cover two essentially different things. Its application, therefore, is contrary to scientific usage. Since too, Kellner meant by "starch equivalent" "starch equivalent for production," why not call it so? Whether the "production starch equivalent" is determined directly, or whether it is calculated from digestible nutrients and "value" number, seems to be beside the point, and immaterial to the discussion. Once it is realised that Kellner's use of "starch equivalent" means "starch equivalent for production," the most serious defect complained of in Kellner's system disappears, since there will exist no longer any danger of the student or scientific worker using the "starch equivalent for production" of a food to express the "maintenance starch equivalent."

That the distinction between the requirements for maintenance and the requirements for fat production when expressed as "starch equivalent" and "value" number in both cases is a rather subtle one, is shown by the fact that Murray, while pointing out the fact that Kellner himself erred, failed to realize at which end of the scale Kellner erred, and thereby unknowingly dropped into the same trap that Kellner prepared for himself. It is unfortunate that Murray's authorities which he quotes should have favoured his point of view, since a consideration of the original data on which they were based would have shown him clearly that they were incorrect, and that Wood and Yule's estimate of 6·35 lbs. starch equivalent for maintenance per 1000 lbs. live weight of animal is a correct one. In other words, when Kellner[1] expressed the energy requirements of oxen at rest in terms of "starch equivalent," he found that the maintenance requirements of the ox were satisfied when 6 lbs. starch per 1000 lbs. live weight were given, and thus the "specious similarity" with Wood and Yule's figures is explained. Later on when he came to collect his data in the Appendix he evidently assumed the starch equivalent so obtained to be the dynamic portion only of the food, and, reckoning the fodder as hay, allowed more thermic energy for maintenance than was required. A critical examination of the original data upon which the figures were based shows this.

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[1] Kellner, *Landw. Versuchs. Stat.* **53**, p. 12.

Daily requirement of metabolisable energy for an ox of 632 kg @ 15·2° C. (average of experiments) = 13,470 cals[1]
Calculated for a 1000 lbs. animal = 10,840 cals.
On the assumption that 1 gr. starch yields 3·76 cals. available for maintenance of the animal body = 6·35 lbs. M.S.E.
On the digestibility figures given by Kellner for medium meadow hay (100 lbs. hay–43·5 lbs. maintenance starch equivalent) – 14·5 lbs. hay.
containing 12·4 lbs. dry matter,
·67 lb. dig. crude protein,
·08 lb. dig. crude fat,
and 5·3 lbs. dig. N-free extract.

From the above-mentioned maintenance requirement of 10,840 cals. Kellner gives 6 lbs. S.E. in his appendix. Calculated as above this would represent 13·8 lbs. hay. Calculated from starch equivalent assuming it to be production starch equivalent (100 lbs hay 23·7 lbs. P.S.E. and 43·5 lbs. M.S.E.) this would represent 25 lbs. hay containing:

21 lbs. dry matter,
·8 lb. dig protein,
·15 lb. dig. fat,
and 9·1 lb dig. N-free extract.

The appendix gives 15 21 lbs. dry matter, ·6 to ·8 protein, ·1 fat, and 7·5 to 9·5 lbs. N-free extract. The error Kellner committed was therefore the reverse of what Murray assumed.

The earliest investigations with regard to maintenance rations of oxen at rest were due to Henneberg and Stohmann[2] who carried out a series of investigations at Weende Experiment Station on two mature draft oxen, weighing 516 and 575 kg. respectively. These animals were fed on various diets containing two or more of the following foods: oat straw, mangels, rape cake, clover hay and rye straw, and sufficient food was given to maintain the animals without change of body weight. In each experimental period, which varied in length from ten days to two months, a collection of faeces and urine was made during the last three weeks of each experiment, and an analysis was conducted on the excreta produced during the last three days in order to arrive at some idea of the digestibility of the foods fed. As a result of these experiments Henneberg and Stohmann came to the conclusion that the requirements of adult oxen of 1000 lbs. live weight were satisfied with a ration containing ·57 lb. digestible protein and 7·4 lbs. digestible N-free extract, *i.e.* an available energy intake of about 13,830 cals. per day. It should be remembered that these experiments lasted for short periods only, and no respiration calorimetry control was used to check the accuracy of the figures obtained by merely weighing the animals. Moreover, in these experiments, no estimation of the fat of the foods was determined except in the case of the rape cake. The figures given, therefore, must be accepted with a certain amount of reserve.

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[1] Kellner, *Landw. Versuchs. Stat.* **53**, p. 13.

[2] Henneberg and Stohmann, Wolff's *Farm Foods*, p. 229; Henneberg and Stohmann. *Beitrage zur Begrundung einer rationellen Fütterung der Wiederkäuer*, Part I. pp. 17-188.

Henneberg[1] records two instances, (*a*) at Weende, and (*b*) elsewhere, of full-grown oxen fed throughout the winter months under conditions of ordinary agricultural practice upon the following diets:

In lbs per 1000 lbs. live weight

(*a*)	(*b*)
12·9 straw	16·3 barley straw
7·1 sainfoin hay	0·4 aftermath
0·4 bean meal	2·0 clover hay
0·4 rape cake	1·3 pea straw
	2·9 mixed barley and oat meal
containing	containing
1 lb digestible protein	7 lb. digestible protein
7·8 lbs nitrogen-free extract	8·8 lbs nitrogen-free extract

The animals fed on ration (*a*) increased 70 to 90 lbs. apiece; those on ration (*b*) performed light work without losing condition. That is to say, a diet containing ·7 lb. digestible protein and 8·8 nitrogen-free extract per 1000 lbs. live weight is more than sufficient to maintain the animal, and Henneberg's and Stohmann's estimate, and in consequence Wolff's estimate, are considerably in excess of the actual maintenance requirements of oxen. Wolff[2], as a result of critical examination of the Weende experiments, comes to the conclusion that as these experiments were conducted at a temperature of 62° to 69° F. the allowance made by Henneberg and Stohmann is rather too small, and he suggests therefore replacing it by a ration containing ·7 lb. digestible protein and 8·4 lbs. digestible nitrogen-free extract per 1000 lbs. live weight. This is the figure given by Wolff in the appendix and quoted by Murray in support of his arguments.

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[1] Henneberg and Stohmann, *Beiträge zur Begrundung einer rationellen Futterung der Wiederkäuer.*

[2] Wolff, *Farm Foods*, pp. 229 and 350.

We have already seen that this estimate of Henneberg and Stohmann and Wolff is unsupported by the practical examples given. Considerations of later calorimetry experiments support the conclusion that the above estimate is much in excess of the normal maintenance requirements of oxen. Kellner[1] fed an ox of 748 kg. on 9 kg. average meadow hay per day for 15 days. During this period the animal lost about 1 lb. weight a day, so the diet was evidently just under maintenance. For a 1000 lbs. ox this diet works out at 12 lbs. hay a day (about). The estimate of 6·35 lbs. starch equivalent for maintenance of Wood and Yule corresponds to 14 lbs. meadow hay, which agrees well with this experiment. An isolated experiment, however, is a dangerous basis for conclusions, and this becomes more evident when the figure quoted by Murray of 17,320 cals.[2] as the energy requirement for maintenance of an ox of 638·1 kg. is considered. This figure is derived from an experiment conducted by Kellner on an "ox B," whose energy requirements for maintenance were extraordinarily high owing to its uneasiness and general restlessness in the calorimeter and while under experiment. So unsatisfactory a subject did this ox prove for experimental purposes that Kellner[3] himself omitted it when making the average on which Wood and Yule's graph is based.

A notable advance in the state of our knowledge with regard to the maintenance requirements of oxen was made when Kühn[4], in a series of painstaking and careful experiments with seven oxen extending over a period of seven years, came to the conclusion that the maintenance requirements of grown oxen are satisfied with a ration containing ·7 lb. digestible protein and 6·6 lbs. digestible nitrogen-free extract per 1000 lbs. live weight per day.

In these experiments, a coarse fodder formed the sole source of the available energy (meadow or clover hay and oat straw) and the experiments included both determinations of digestibility and the total metabolism of nitrogen and carbon including the excretion of methane.

The death of Kühn left the investigation in the hands of Kellner[5], who, as a result of experiments with two oxen A and B and combining some of Kühn's earlier results, came to the conclusion that an ox of

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[1] Kellner, *Landw. Versuchs. Stat.* 1898, **50**, p. 245.

[2] Goodwin, *Scientific Feeding of Animals* (Kellner's), p. 50.

[3] Kellner, *Landw. Versuchs. Stat.* 1896, **47**, 275; **50**, 273, footnote.

[4] Kühn, *Landw. Versuchs. Stat.* 1894, **44**, 257–581.

[5] Kellner, *Landw. Versuchs. Stat.* 1896, **47** 275.

630·3 kg. live weight at 15·5° C. requires 15,167·5 cals. per day for maintenance, *i.e.* a 1000 kg. beast requires 24,000 cals. per 24 hours. This figure was obtained by averaging Kühn's oxen (II, V, VI, and XX) with ox A, ox B not being included for reasons already given above. Calculating this average on the basis of Rubner's surface law, the figure per 1000 lbs. live weight = 12,200 cals.

Further experiments with three oxen previously fed on a fattening ration led Kellner[1] to conclude that an 800 kg. animal in a fattening condition required rather more for maintenance per day than an animal of similar weight in store condition, namely 24,900 cals. per 1000 kg. per day. The advent of Rubner's surface law, and consideration of the fact that the energy calculated from an addition of flesh or fat represented roughly only 60 per cent. of the metabolisable energy of the food digested, caused Kellner[2] to recalculate on this basis the maintenance requirements of an ox at rest. From the results thus obtained Kellner constructed the following table:

Maintenance Ration.

Live weight	Calories	Digestible organic substance Meadow hay.
Observed. Average of experiments:		
632 kg.	13,470	3·85 kg.
Calculated from surface law:		
450 kg.	10,740	3·07 kg.
500	11,520	3·29
550	12,280	3·51
600	13,010	3·72
650	13,720	3·92
700	14,420	4·12
750	15,100	4·31
800	15,760	4·50

This represented an energy requirement of about 10,840 cals. per day per 1000 lb. ox, *i.e.* a maintenance starch equivalent of 6·35 lbs. a day. It is this figure that Wood and Yule read off their graph, and it is from this table that Kellner calculated the figures from which Wood and Yule's graph is derived. This estimate is, however, inconsistent with the figure given in the appendix[3] to Goodwin's translation, since the ration there suggested represents an energy requirement of

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[1] Kellner, *Landw. Versuchs. Stat.* 1898, **50**, 245.

[2] Kellner, *Landw. Versuchs. Stat.* 1900, **53**, 1–474.

[3] Kellner, *Scientific Feeding of Animals*, p. 392.

14,500 to 18,350 cals. per 1000 lb. live weight per day, and is practically identical with that given in the appendix to Wolff's *Farm Foods.* We must, therefore, assume either that Kellner copied the figures straight from Wolff's table, or that he made the mistake of reckoning the amount of hay required from the starch equivalent for production instead of from the total metabolisable energy of the food. In the latter case, the mistake, the existence of which Murray recognises, is the exact reverse of that which Murray assumes, and Murray's estimate of the energy requirements of oxen at rest is much too high.

Independently of Kellner's later experiments, a long series of digestibility experiments was conducted by Armsby[1] in America on cattle. These experiments, which were of long duration, and in which the animals were weighed very frequently, support in their findings the results given by the more exact calorimetry experiments of Kellner. Kellner's experiments are open to the objection that the results given are based on an assumption in most cases of the thing Kellner was endeavouring to find, *i.e.* an exact knowledge of the maintenance requirements of an ox at rest. Where the animals used for the estimation of the average had put on weight (and five out of the seven animals used for the average had increased in weight during the experiment), Kellner added a certain percentage of energy to the total stored; since he had found in previous experiments that of 100 parts available energy only 43 parts in the case of meadow hay, and 37·6 parts in the case of oat straw, were available for fat production. But these figures were arrived at by assuming the maintenance requirements of an ox at rest to be 21,300 cals. per 1000 kilos. live weight. In other words Kellner was guilty here of the vicious practice of arguing in a circle. The difference is not large, and does not affect Kellner's estimate to a large extent, but it is essential to realize here that Kellner's estimate of the maintenance requirements of an ox at rest is not an exact one. As a result of Armsby's investigations into the maintenance requirements of cattle, the following estimate was arrived at, and represents the average of 12 different experiments. The food fed was coarse timothy hay, and the energy of the digestible food was estimated by means of the Berthelot bomb calorimeter. As a result of these determinations Armsby came to the conclusion that the energy requirements of a 500 kg. ox are satisfied with a total digestible energy of 12,771 cals. at 51° F., or 11,980 cals. per 1000 lbs. live weight.

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[1] Armsby, *Bulletin* 42, Penn. State Coll Expt. Stat. 1898.

From Kühn and Kellner's results, and the experiments of Armsby, it is evident that the estimate given by Wolff and the older authorities, and included by Kellner in his appendix for the maintenance of a 1000 lb. ox at rest, is much too high, and that the energy requirements of an ox of this weight are satisfied on a diet supplying ·6 lb. digestible protein and 10,840 cals. per day, *i.e.* a maintenance starch equivalent of 6·35 lbs. per day. In other words the "generally accepted ration" of 20 lbs. hay per day for a 1000 lb. ox is much too generous for maintenance purposes and should be replaced by a ration of 14 lbs. hay per day. Murray's criticism of the value to be attached to the conclusions reached by Wood and Yule in their paper consequently becomes pointless. The following table summarises the estimates of various authors of the maintenance requirements of an ox at rest, the results being calculated in every case to the needs of a 1000 lb. ox.

Maintenance Requirements of Oxen.

Author	Maintenance starch equivalent	In calories per 1000 lbs. L.W.
Henneberg and Stohmann	8·1	13,830
Wolff (Appendix)	9·2	15,700
Kellner (Appendix)	9·6	16,380
Kühn	7·5	12,850
Kellner (1896)	7·1	12,200
Armsby (1898)	7·0	11,980
Kellner (1900)	6·35	10,840

With regard to the graph, the data derived from *Die Ernährung* on which the graph is based certainly depend upon the application of Rubner's[1] surface law, and the data given were calculated by Kellner[2] on this basis. But the figure from which they were calculated is an average of experiments of long duration on seven oxen, and this estimate of the energy requirements of an ox, namely 13,470 cals. for maintenance of a 632 kg. ox, at 15·5° C. or 10,840 per 1000 lbs. live weight, is an experimental determination. The truth of Rubner's surface law, derived from experiments on dogs (presumably adult) varying from 3·2 to 31 kilos., is freely accepted by Murray. While in no way criticising the accuracy of Rubner's results, or the universal applicability of this law, the possibility of a modification of our ideas on this subject in the light of future research must clearly be borne in mind. Rubner's law states that the energy metabolism is proportional to the superficial area of the animal. In other words, the metabolism varies as the amount of heat loss at the surface, and its variation in accordance with this law is necessary for the maintenance of a constant temperature. When Rubner first established this "law of surface area" he explained its constancy on the assumption that the loss of heat from a body must depend upon the extent of the surface, and stimuli received from that surface determined the amount of metabolism and so maintained the body temperature while allowing for loss of heat by radiation. But at a temperature of 30° C. where all possibility of thermal stimuli was removed, Rubner found in the case of two guinea-pigs of different sizes that the law still held, and the explanation that the variation in metabolism of different animals was due to the "chemical regulation" brought about by specific sensory influences of cold proceeding from a definite area of surface, broke down. The insufficiency of the explanation was realised by Rubner who, however, stated that even at this temperature the law is still a necessity if the general mechanism for loss of heat in the various animals is the same in all. From a physiological standpoint, it is difficult to accept this explanation. The metabolism of a body must depend upon the mass of living cells it contains, and the temperature of this mass, and the mechanism for the regulation of heat loss and the maintenance of a constant temperature, must therefore be a subsidiary one, and not the determining factor of metabolism.

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[1] Rubner, *Zeitschrift für Biologie*, 1883, **19**, 535.

[2] Kellner, *Die Ernahrung Land. Nutztiere*, p. 395.

Each living cell must possess a basic requirement of energy at a given temperature for its needs, and the heat produced as a result of its activity must be a secondary result. The chemical regulation consequently is only required when the heat so produced is insufficient to provide for loss by radiation, etc., and maintenance of the cell at its normal temperature. That this is so is supported by the fact that where the food given is more than sufficient to supply the energy requirements of the body, chemical regulation is not brought into play until the temperature of the surroundings has fallen to the point where the energy requirements supplied by the food are insufficient to satisfy the body's requirements. Moreover, the path of heat loss varies considerably according to the temperature of the environment. Thus, whereas in a dog at low temperature the path of the heat loss is through radiation by the skin, at a temperature of 37° C. the path is by means of evaporation of the water from the lungs, and is here independent of the surface.

The normal energy metabolism of an organism as measured by the heat loss consequently covers two things, (1) the basic energy requirement of the living cell for the adequate expression of its activity, which requirement would be independent of the body surface and only a function of the temperature of the cell, and (2) the energy lost through difference of temperature of the animal and its surroundings, and depending obviously on the extent and nature of the surface. Where the temperature difference between the animal and its surroundings is such that all loss due to the second influence is cut out, we should expect the energy requirement to be proportional to the body weight rather than to the surface.

The data upon which Rubner's law was established are based on calorimetry experiments on dogs whose weights varied from 3·2 to 31·2 kg. Leaving out the two dogs of smallest weight the results obtained could be expressed equally well as a straight line. In other words, between 10 and 30 kilos., the differences between the energy requirement expressed as a linear function of the body weight and the energy requirement expressed as a linear function of the body surface are within the limits of experimental error. This is shown much more clearly when we deal with the data for oxen. As expressed in terms of starch equivalent for maintenance, the values calculated for oxen of different weight by means of Rubner's surface law form such a flat curve between the weights taken, that Wood and Yule found it sufficiently accurate to express these results by a straight line. Therefore, for oxen between the weights taken the maintenance requirements are, within the limits of experimental error, a linear function of the body weight, and may be expressed by the simple graph given. Murray, in his paper, proposes to express this perfectly simple relation between body weight and energy requirement by the somewhat complicated formula

$$E = \tfrac{2}{3} \log M - 1{\cdot}19723$$

where M = live weight of animal and E = maintenance starch equivalent.

This formula is obviously not based upon the graph given but upon consideration of Rubner's law of surface area, and the close relation between the results calculated from Murray's formula and the figures taken from the graph emphasize the sufficiency of the simple straight line graph of Wood and Yule for the expression of the maintenance requirements of oxen between the weights shown. As calculated by Murray, the concordance is as follows:

M	800 lb.	1000 lb.	1200 lb.	1400 lb.	1600 lb.	1800 lb.
E (graph)	5·5	6·35	7·2	8·0	8·8	9·4
E (formula)	5·47	6·35	7·17	7·95	8·69	9·4

Emphasis has been laid on this last point, since it is difficult to see how a formula such as Murray gives is going to appeal to a farmer who has already rejected Kellner's starch equivalent system on account of its complexity of application. The formula may be more scientifically accurate, and Murray should be given the credit he deserves in evolving *a formula which satisfies alike the maintenance requirements of two such widely different animals as a young growing pig of* 50 lbs. weight and an adult ox of 1800 lbs.; but at the same time it is felt that with regard to oxen the simpler mathematical relation given by Wood and Yule is more within the region of practical politics, and its use is thereby justified. There is much that is valuable and stimulating in Murray's paper, and the fresh aspect he brings to bear on the science of animal nutrition gives one much food for thought. Much of the criticism he brings to bear on the starch equivalent system, however, loses greatly in value when the paper of Wood and Yule is taken into consideration, since these authors show how many of the faults adherent to the Kellner system may be rectified. The beauty of the Kellner system lies in the fact that it gives the comparative values, and not the absolute values, of feeding stuffs for fattening purposes, and much careful thought should be devoted to consideration of its values and defects before deciding to substitute for a simple quantitative number mathematical formulae of varying complexity, which, though they may be scientifically more accurate, are not likely to arouse much enthusiasm among the farming community. The farmer desires to know, not how much fat or milk or work a food will produce, but rather which of several foods is more economical for any purpose he has in view. And it has yet to be proved that the starch equivalent system is incapable of giving him the right interpretation with regard to this point

(*Received May* 11*th*, 1915.)

VARIATION IN THE MALE HOP, *HUMULUS LUPULUS* L.

BY H. WORMALD, A.R C.Sc., D.I.C., B.Sc. (Lond.).
(*Research Department, South-Eastern Agricultural College, Wye.*)

THE hop plant *Humulus lupulus* L. is best known in Britain from its cultivated forms grown for use in the brewing industry. Those varieties which are grown on a commercial scale in this country all conform to the general description of *Humulus lupulus* L. and the presumption is that they are all variations (directly, or indirectly through other varieties) from the female form of the original wild hop. Since the plant is dioecious it follows that any one of these varieties was in all probability derived primarily from *one ♀ plant* which arose either as a mutation or as a hybrid and has subsequently been propagated vegetatively by cuttings or "sets," *i.e.* the variety is represented by a number of plants comprising a *clone*[1]; such a variety is not necessarily provided with a corresponding male form possessing all the vegetative characters of that particular ♀ variety.

The varieties of the female hop are, at the present day, very numerous but no attempt has hitherto been made to distinguish varieties of the male plant, for, as Prof. Percival points out[2], "on account of their being of no use to the grower, males have never been subject to special selection and improvement."

The value of the male hop to the practical hop-grower in this country was, until a few years ago, a subject of much debate. Recent observations and experiments carried out at Wye College by Mr E. S. Salmon and Mr A. Amos have proved conclusively, however, that for the production of "well grown-out" hops of the best English varieties the development of a certain number of seeds is of primary importance[1]. This involves pollination, for there is no definite proof that parthenogenesis[2] obtains in the hop. Hop-growers therefore are now strongly advised to plant a certain proportion of male hops in their hop-gardens, and that this advice is being followed is shown by the numerous applications, made by farmers of Kent, Surrey, and Sussex, for the male-hop "cuttings" supplied free by Wye College[3].

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[1] The word "clone" was first used by Webber in 1903 and has been adopted by Dr Johs. Schmidt, Director of the Carlsberg Laboratory, Copenhagen. In his recent paper "On the Aroma of Hops" (*Comptes-rendus des travaux du Laboratoire de Carlsberg*, 11me Vol. 1915) Dr Schmidt, in discussing the use of the word "clone," writes (*loc. cit.* footnote, p. 153): "I would suggest that the word be adopted into the terminology relating to hops, where such a term is actually needed. A hop-clone would thus be all those plants derived from the same seedling by vegetative propagation, a clone-plant being any single plant belonging to the clone."

[2] *Agricultural Botany*, 4th Ed. 1910, p. 345.

In order to meet the demand for cuttings some 200 "hills" of male hops are now established in the nursery attached to the hop-garden on the College farm. An examination of the plants shows that they vary considerably in some of their characters, but of these the only ones taken into consideration as being useful to the commercial grower were (1) *Time of flowering, i.e.* early, mid-season, or late, since a male hop to be of any value for pollination purposes must necessarily be in flower with mature pollen at the time that the female plant has receptive stigmas[4]. (2) *Vigour,* including the suitability for growth on certain soils; a male hop that thrives in East Kent may not be successful when grown on the heavy soil of the Weald.

Breeding operations now being carried out by Mr Salmon for the purpose of raising new varieties suggested the advisability of obtaining descriptions, as complete as possible, of those plants used in the "crossings," particularly of the males since there are no specified varieties of that form. It was proposed therefore that the writer should study and keep under observation for a number of consecutive seasons a large number of male hops, in order to determine those characters, if any, which could be taken as a basis for a classification (not necessarily a *natural* one) and identification of varieties of male hops.

Of the hills available 120 were selected as being suitable for detailed observation. Among those chosen fourteen plants[5] were each represented by three hills (*i.e.* two of the hills were obtained by planting cuttings taken from the third, so that all three are of the same clone in those cases where the original hill is known to be a seedling), and seven by two hills each, similarly obtained. The remainder are not known to have any direct vegetative connection with each other in their own generation, and it may be that each is the sole representative in the garden of a seedling plant; they include, however, four raised from cuttings obtained from *Oregon* which are indistinguishable from one another. The number of individual seedlings represented in the hills selected is therefore reduced to about 80, including 53 which have been raised as seedlings in the College nursery The advantage derived from having two or three hills of one plant in different parts of the garden is, that the limits of variation in those characters which are influenced by environmental factors can be determined with a greater degree of accuracy.

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[1] *The Journal of the South-Eastern Agric. College,* No. 17, pp. 364–391.

[2] I.e. *Somatic Parthenogenesis* of Winkler or *Parthenapogamy* of Prof. Farmer.

[3] *The Journal of the South-Eastern Agric. College,* No. 21, p. 425.

[4] When the stigmas of the ♀ flowers are receptive they project from between the bracts and bracteoles of the strobiloid inflorescence which develops into the "hop" of commerce, and the plant is "in burr."

[5] Owing to an attack of "nettle-head" disease only eleven of these were fully available for the season 1914, since two of the three hills in one case and one hill each in two others had to be "grubbed" during the winter 1913–14.

Prof. J. Percival in his *Agricultural Botany* writes[1]: "It is somewhat curious that, although female seedlings show considerable variation, we have never seen any morphological differences among males, no matter what their origin, except in one or two solitary instances where the 'bines' were a paler colour than usual." Reference has already been made to these words by Mr Salmon, who says[2]: "This statement is somewhat misleading, since we find in the forms, or varieties, of the male hop quite as much variation in such characters as the colour of the stem and petioles, length of the lateral branches, and in other vegetative characters as in the female hop-plant." The object of the present paper is to present a detailed account of observations which warrant this conclusion.

The hills examined (120 in number and representing, as noted above, about 80 plants) showed variation in the characters set out below. The investigations have extended over three seasons, so that in some cases (*i.e.* where one individual plant has been grown in three hills) it has been possible to make nine observations of any one particular character. The system of training the hops adopted in the nursery where all these males are grown is that known as the "Butcher System[3]."

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[1] *Loc. cit.* 4th Edit. 1910, p. 346.

[2] *Journal of Genetics*, Vol. III. No 3, Feb. 1914. Footnote on p. 195.

[3] A description of this system of training hops is to be found in an article on "Hop Cultivation" by Mr A. Amos in the *Journal of the Board of Agriculture*, Feb. 1910, Vol. XVI. with figures on p. 891.

The male hops examined were found to vary in the following particulars:

Time of flowering.
Stem (bine).
  Colour: green, red, or intermediate.
  Ridges: rough or smooth; darker or lighter than the general colour of the stem.
  Length of internodes.
Leaves. Total length of petiole and lamina.
  Lamina: colour, dark or light green; flat or margins of lobes recurved towards lower surface; wrinkling of the leaf feeble or well-pronounced; number and shape of lobes; glands on lower surface numerous or few; size of glands.
  Petiole: colour of upper and lower sides; roughness of lower (dorsal) surface; depth of furrow on upper (ventral) surface.
Laterals (inflorescences).
  Length: absolute length and relative to subtending bract.
  Number of nodes at which bracteoles are leafy.
  Length of internodes.
  Length of secondary laterals.
Stipules: upright, spreading or recurved.
Flower.
  Perianth segments: dimensions of segments and number of glands on outer surface.
  Anthers: number of glands in the outer (dorsal) furrow.
  Disc: number of glands present.

In order to secure uniformity in preparing a record of observations the following plan was adopted:

The *Time of Flowering* is understood to be the first date when open flowers were seen; this was selected rather than the date when the plant was in full flower as being more easily and accurately determined. The remaining characters were taken when the plant was well in flower.

The colour of the stem was determined for that portion of the bine from the ground to the "breast-wire[1]," and the roughness of the ridges taken for a short length (about 2 feet) just above that wire.

For observations of the leaf-glands two leaves were taken from each hill, one at the level of the breast-wire, the other midway between that and the top wire.

The measurements of the laterals were taken at two levels, the first at one-third the distance from the breast-wire to the top wire, and the other at two-thirds that distance; the general observations on the leaves[2] and the length of the internodes of the bines were taken between those two levels.

[1] The "breast-wire" or middle wire of the Butcher System is about 4 feet 6 inches from the ground.

[2] Or rather *bracts*, since the laterals (inflorescences) grow in their axils.

Time of Flowering.

The records taken were the dates on which flowers were first seen open. Usually a hill will be in full flower a week or 10 days later and may remain in flower for still a fortnight longer. The period during which a plant remains in flower is modified by atmospheric conditions; a warm dry air favours the dehiscence of the anthers, while cold, moist air is unfavourable. The time of flowering for any individual varies with the age of the plant, and probably to some extent with the season (the dry summer of 1914 hastened the average time of flowering of the mature plants, though the difference was practically negligible).

The older males in the garden, *i.e.* the well-established seedlings and the plants raised by cuttings from them, represent 87 hills, and it was found that in each of three of these the date of flowering had been the same for the years 1912 and 1913; 44 had flowered earlier and 40 later during the second year, with an average date of but 0·29 of a day earlier, which seems to indicate that the seasonal variation in the flowering period for the two years was practically nil. In 1914 the average time of flowering for these mature plants was 1·6 days earlier than in 1912. Thus it will be seen that, though certain individuals may show considerable variation from one year to another, the mean time of flowering throughout the garden was, for the well-established plants at any rate, about the same for the three years, and the seasonal modifications are probably slight except during abnormal seasons.

One of our most promising early ♂ hops is one labelled Z 12 [= 292 and 293][1], which in nine observations has commenced to flower on dates ranging from June 29th to July 8th with a mean date July 3rd. Another early one is 294 [= 295] with time of flowering June 30th to July 4th with a mean date July 2nd.

Late males include E 16 [= 44 and 48], which in nine observations came into flower from July 23rd to August 6th, with July 28th as the mean date. A 13 [= 315 and 316] may be mentioned as an example as a midseason male hop: its date of flowering is July 7th to 17th with July 12th as the mean.

These observations are evidence that, just as there are early, midseason, and late varieties of female hops, so also the same variation may be taken as a characteristic varietal feature of the male hops.

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[1] The numbers in brackets refer to hills obtained by planting cuttings from the hill bearing the number in front of the bracket.

Stem (*Bine*).

Colour. Although the colour of the bine varies from a pale green to a dark red among the plants under observation, the variation in colour in the individual plant is very slight, in fact, among the English forms of male hops this character appears to be more constant than any other, persisting from year to year in the same hill and in different parts of the garden for hills having the same origin. This is not only true for the pale green and the dark red stems but also for the intermediate forms; in the latter the colour is not generally a uniform intermediate shade between green and red, but consists of a mottling of small areas alternately red and green. This mottled appearance is often very marked and may persist to some extent in the green bines and the red bines. In the palest green and darkest reds the mottling is not visible but when these extremes are departed from small red dots in the one case and small green dots in the other appear. In others again the dots are more conspicuous and there is either a green ground-tint with red markings or a red ground with green markings and the general appearance approaches more nearly the intermediate mottled form.

The coloration of the petiole is associated with that of the stem and usually an observation of the petiole of a leaf removed from a plant is sufficient to determine approximately the colour of the stem from which it was taken. This association of colour will be treated more fully when dealing with the petiole.

Ridges. The hop-bine is provided normally with six ridges[1] corresponding in position with the leaf-trace bundles of the vascular cylinder. In colour the ridges are almost invariably darker than the surface of the stem between the ridges. The pale green bines have ridges of a rather less pale green and the red bines have ridges of a darker red except in the very dark ones where the tendency is towards an almost uniform deep red. Occasionally the ridges may be seen to be paler than the general surface, but in such cases transitions to dark ridges will almost certainly be found on the same bine. No case has been observed where the ridges are *consistently* paler than the rest of the surface and only one plant was noticed in which they are *usually* so; this plant, J 36, is a seedling (raised from the German variety "Stirn," the male parent being *probably* Z 12) producing a mottled bine with ridges generally green (but sometimes with transitions to red) and this characteristic has appeared in each of the past three seasons. Sometimes the darker colour of the ridges is very pronounced and the bine in consequence is conspicuously striped.

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[1] Occasionally a bine with nine ridges and bearing a whorl of three leaves at each node is met with.

The ridges are beset with small wart-like emergences each bearing a T-shaped spine at its apex, the cross-piece of the T being parallel with the longitudinal axis of the stem and acutely pointed at each extremity. When these "hairs" are numerous the bine is harsh to the touch, when they are few in number it may feel almost smooth, but no plant has been met with where they are absent altogether. Whether the degrees of roughness can be accepted as characters to be used in defining varieties is not yet certain, since individuals are often by no means constant in this respect. Some plants, however, are appreciably rougher, others smoother than the average and retain the character from year to year.

The length of the internodes is probably influenced too much by the weather, method of training, general vigour and age of the plant to be of any value systematically, unless indeed vigour itself is to be looked upon as an inherited character[1]. The internodal measurements were recorded by taking the minimum and maximum lengths occurring in the middle third of the distance between the breast-wire and top wire. The internodes may vary from 8 to 13 inches even in the same mature plant; usually the variation is from 9 to 12 inches. In four hills raised from cuttings obtained from Oregon in 1908 the variation during 1912 and 1913[2] was from 10 to 13 inches, and an average of the 16 measurements taken (*i.e.* the minimum and maximum for each of the four hills in both seasons), which may be taken as the approximate mean length of internode, is 11·45 inches; for comparison with this 50 readings for English ♂ hops (25 minima and 25 maxima) were taken at random and the average was found to be 10·48 inches or approximately one inch less than the average for the Oregon males. This suggests that some varieties may possess a factor for long internodes, but data to hand are insufficient for a definite statement on this point. It may be interesting to note, however, in this connection that three plants (ref. nos. K 1, N 52, and O 12), each of which is a seedling of a Canterbury Whitebine *crossed with an Oregon ♂ hop*, have produced internodes 14 inches in length.

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[1] Vigour in a seedling is probably often due to the stimulus resulting from fertilization.

[2] Unfortunately three of the hills succumbed to "nettle-head" disease and had to be removed before observations were resumed in 1914.

Leaves.

The length of the leaves (bracts) varies from 7 to 10 inches, rarely is 11 inches reached; the variation appears to be wholly "continuous," depending on the age and vigour of the plant and on the environment.

Colour of the leaves. The range of tint in the leaves is not extensive, though there is evidence that some plants with leaves paler than the average retain that character to a greater or less extent from year to year and when transplanted as cuttings to other parts of the garden. To determine this point absolutely may involve the use of a colour chart.

Lamina. The lamina of the leaf may be either almost flat or the margins may be more or less recurved so that the upper surface is convex, and the irregular wrinkling of the leaf, due to the relatively more vigorous development of the tissues between the veins, may be pronounced or feeble. A distinct wrinkling of the surface is usually associated with a strong recurving of the marginal portions of the leaf. The constancy of this character is, like the last, at present doubtful, yet observations tend to show that at any rate the extreme types are fairly constant.

The four Oregon male hops already mentioned have borne leaves, during the time they have been under observation, which are decidedly less recurved and wrinkled than a typical English male hop. Two English ♂ hops, D 22 [= 325 and 326] and F 2 [= 309 and 310], show the same character, all the six hills having produced leaves weakly wrinkled and recurved during the last three years without exception. Several single hills have shown the same tendency during the same period.

Some hills, relatively few in number, bear leaves which are more strongly recurved and wrinkled than the average. The plant showing this feature most conspicuously is No. 279, which is a very vigorous seedling, raised from the Fuggles crossed with an English male hop, and was planted out in the garden in 1908; only one other hill has hitherto been raised from it (viz. No. 336) and this has not been long enough established for its characters to be constant.

The lobing of the leaves. The first leaves (succeeding the cotyledons) produced by a seedling hop plant are cordate and toothed but unlobed; later three-lobed and then five-lobed leaves appear. In the English hop the five-lobed character is retained in the mature plant, the majority of the leaves of any one hill being of that form, while three-lobed and seven-lobed leaves are comparatively of rare occurrence under the conditions of growth which obtain in the garden where these observations were made. Only one plant (No. 39) has been noticed on which the leaves are more frequently three-lobed rather than five-lobed; this feature was particularly noticeable during the past season (1914), and since the individual was planted out in 1909 it is probably a characteristic of this plant, as others of the same age and growing in the immediate neighbourhood produced five-lobed leaves.

In the Oregon hops, however, the number of lobes varies from 5 to 11, the terminal lobe of the English form being often represented by three lobes. The lobing of the leaves of these Oregon plants differs from that of the usual type in other respects. The lobes are more acuminate and the sinuses between the lobes deeper; as a result of this the lobes themselves are relatively longer. Thus the length of the terminal lobe is about three-quarters that of the whole lamina, the length of the lobe being about twice its breadth, and the width at the base (*i.e.* the distance between the two sinuses) is about half the breadth of the lobe. The corresponding dimensions for an English hop are: length of terminal lobe about two-thirds (usually less) length of lamina; breadth about *equal* to length; base *more* than half the width of the lobe.

Glands on the leaf. On the under surface of the leaves are sessile, capitate, glandular hairs, often just visible to the naked eye but easily seen with a lens. They bear a great resemblance to the lupulin glands found on the bracteoles of the ♀ inflorescence. The hop owes its bittering property to the resins secreted within the lupulin glands, and it is probable that the glands on the leaves also secrete bittering resins, since it is recorded[1] that hop leaves have been used for brewing.

To determine the variation in the number of glands on leaves of different plants it was found necessary, in order that the results should be comparative, to select portions of the leaves corresponding in position and in area. The method adopted was as follows: a circle

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[1] Braungart, in *Der Hopfen* (München u. Leipzig, 1901), p. 170, writes: "Merkwürdig ist, dass man die Laubblätter des Hopfens eben wegen dieser Drüsen in Hopfennotjahren (getrocknet) schon zur Verwendung in der Brauerei, besonders zur Herstellung geringer und Nachbiere, empfohlen hat."

Prof. R. Bradley, of Cambridge University, in *The Riches of a Hop-Garden Explained* (London, 1729), wrote: "It often happens by haste, that the smaller leaves of the plant mingle with the hops. At the time of stripping these leaves are of good virtue, and were alone sold in Flanders, Anno 1566, for twenty-six shillings and eight pence a hundred, no one hop being mingled with them."

¼ inch in diameter was punched through a piece of thin sheet metal about 2 inches square; this metal plate was so placed on the leaf under examination that the circle came midway between the sinus at the base of the terminal lobe and the junction of the petiole and lamina. By means of a pocket-lens magnifying 10 diameters, the glands as a rule can easily be counted; when the glands are numerous the smaller veins are often of service, since they divide the circle into smaller areas which can be taken in order. In this way two countings could be made for any one leaf, one on each side of the mid-rib. Two leaves were selected from each hill, one at the level of the breast-wire and the other midway between that wire and the top wire; thus four readings were taken for each hill each year. In each of eleven cases 36 countings have been possible, *i.e.* where three hills have the same origin, and the general conclusions arrived at are based chiefly on the average obtained from 36 such countings in each case, although in certain typical examples fewer readings were taken, since two hills (or perhaps only one) of each were available.

Although there is often considerable variation in the number of glands borne by the leaves of a single plant, this variation (for a ¼ in. circle as indicated above) is about a certain mean which may be as high as 80 or as low as 20. Usually the number lies between 40 and 60; thus in the eleven ♂ hops from each of which 36 countings were possible the following numbers were obtained:

Reference numbers of hills	No. of glands to ¼ in. circle						
	Lower leaves			Upper leaves			Av. of 36 countings
	Min.	Max.	Av. of 18 countings	Min.	Max.	Av. of 18 countings	
A 13 [–315; 316]	51	106	75	42	94	64	69
B 11 [–298; 299]	30	87	55	33	67	51	53
C 7 [300; 301]	41	88	59	33	65	47	53
D 22 [325; 326]	35	70	51	32	78	47	49
F 2 [–309; 310]	18	51	34	21	36	28	31
E 10 [311; 312]	22	76	44	25	58	37	41
E 16 [44; 48]	32	74	46	25	49	35	41
G 27 [319, 320]	26	78	46	26	65	39	43
Z 12 [–292; 293]	26	64	41	25	52	44	42
30 [–35; 42]	38	102	59	34	64	47	53
168 [–167; 169]	32	151	90	50	101	74	82

A comparison of the above figures shows two features of interest:

(1) In every case, with one exception, viz. D 22 [= 325; 326], the range of variation (difference between minimum and maximum) is greatest for the lower leaves; this was found to be the case also for the great majority of the rest of the plants examined.

(2) With the exception of Z 12 [= 292; 293] the glands are more numerous on the lower leaves than on the upper, as shown by the average of the 18 countings in each instance; this condition again obtained throughout the rest of the garden except in seven plants, where, however, the difference was not considerable as is often the case when the difference is in the other direction as in 168 [= 167; 169] of the above table.

It is advisable therefore when making observations in this connection, to select leaves at about the same level, which, for preference, should be a fairly high one.

Of all the plants examined the one with the highest average is the one appearing last in the table, viz. 168 [= 167; 169], with an average of 82; others with numerous glands are A 13 [=315; 316] as shown in the table, and H 22 [= 26] which has given 73 as the average of 21 countings. The Oregon ♂ hops too have shown a high average, viz. 67, the maximum and minimum numbers obtained being 117 and 50 respectively.

Of those plants producing few glands the most striking are

F 2 [= 309; 310] shown in the table to have an average of 31.
269 [= 297] min. 18, max. 37, av. 28 (for 24 countings).
67 min. 16, max. 31, av. 23 (for 12 countings).

The plant giving the lowest average of all, viz. 16, is K 1, a seedling obtained by crossing a Canterbury Whitebine with an Oregon ♂.

Size of the glands. The glands are easily seen if a fresh leaf or portion of one is placed with the lower surface upwards on the stage of a microscope and examined by the aid of a ⅔ in. objective. It is preferable to use reflected light only, for then the glistening yellow glands contrast well with the dark green of the leaf surface. The diameter of the glands was measured by means of an eyepiece micrometer; during 1912 the ordinary type[1] of eyepiece micrometer was employed, but during the two succeeding years the Leitz's Stufen-Mikrometer was found to be not only much more convenient, but also the divisions of the scale were more easily seen against the dark background of the leaf. In this way it was found possible to ascertain rapidly the diameter of the glands correct to 5 μ. One leaf from each hill was selected and measurements taken for those glands showing extreme variation, the smallest glands which appeared to be not fully developed and those which were not more or less circular not being taken into consideration.

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[1] *I.e.* the type in which a glass circle with scale is dropped into the eyepiece.

The variation was found to be somewhat considerable even on the same leaf; usually it is from 120 μ to 160 μ. No plant was found to have glands consistently large or consistently small, but it was seen that in general those leaves with most numerous glands showed these to be above the average in size. To illustrate this point the following table shows the minimum and maximum dimensions of the leaf-glands for those plants already mentioned as possessing numerous glands and those with few:

Reference number of plant	Av. no. of glands per ¼ in. circle	Diam. of glands	
		min.	max.
168 [-167; 169]	82	130	175
H 22 [−26]	73	130	190
A 13 [=315; 316]	69	120	170
E 12; F 7; F 9; F 17 (Oregons)	67	120	180
F 2 [=309; 310]	31	120	165
296 [−297]	28	120	165
67	23	120	150

That this is not an invariable rule is shown by the fact that occasionally where the glands are few in number they may also reach a size above the average. Two such cases have come under my observation, both plants being hybrid seedlings raised in the nursery; one is a monoecious hop (ref. no. J 2, raised from the German variety Stirn × ♂ Z 12), and the other K 1 to which reference is made above. In each of these the maximum 190 μ has been reached.

The petiole. The most interesting feature connected with the petiole is the colour; this is never uniform, but (with the exception of those petioles borne by the typical pale green stems) varies on one and the same petiole from green to some shade of red. The lower side is invariably green toward the distal end but may show transitions to red towards its proximal extremity. The colour relation between the petioles and the bines on which they grow is as follows:

Colour of stem	Colour of petiole	
	Upper side	Lower side
Pale green	Green, but a little darker than lower side	Green
Green predominating but red marks fairly conspicuous	Green, tinged with red	Green
Typically mottled	Red tinged with green	Green
Red predominating but green marks fairly conspicuous	Red	Green
Dark red	Red	Green at distal end shading off to red at proximal end.

The lower side of the petiole is provided with bifid hairs similar to those of the stem; the petiole, however, is almost invariably rougher than the stem itself. Thus a bine which might be described as "almost smooth[1]" usually has a "moderately rough" petiole, while a "moderate rough" bine has a "rough" petiole.

The upper side is channelled by a single longitudinal furrow of varying depth. In the Oregons it is shallow, and the upper surface of the petiole is therefore almost flat. In the English type the furrow is more or less V-shaped in transverse section (except towards the base, where it usually tends to disappear altogether) but is often very variable even in the same plant, there is evidence, however, that in some instances there is an approach to constancy in one direction or the other. One plant represented by three hills (so making nine observations possible) has shown furrows distinctly V-shaped in section on each occasion and the same feature has been noticed in several single hills, while in others the furrow more nearly resembles that of the Oregon form.

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[1] It should be noted that these terms are merely relative and no attempt has yet been made to reduce them to terms of size and frequency of the hairs.

The Laterals (Inflorescences).

By "lateral" is meant a branch growing in the axil of a leaf (bract) borne on one of the main stems (bines). The laterals above[1] the breast-wire with a few exceptions bear the staminate flowers and are thus the inflorescences. These bear bracteoles in the axils of which develop secondary laterals, which may be opposite or alternate; on long laterals they are usually opposite, on the short ones usually alternate. The utility of a male hop for pollination depends of course on the output of pollen, or, in other words, its value is according to the fertility of the laterals, and an attempt was made to determine the fertility of any plant in terms of the characters of the laterals.

Other things being equal, the longer the lateral the more flowers it bears, but factors which must be taken into consideration are the length of the internodes of the laterals and the length of the secondary laterals. Since as a rule the internodes and secondary branches decrease in length gradually towards the distal end of the lateral, it was considered sufficient to take measurements of the first internode[2] and of the longest secondary lateral.

Other characters, viz. (1) the number of nodes on the lateral at which the bracteoles were leaf-like[3], (2) whether the bracteoles were opposite or alternate, and (3) length of the stipular inflorescences[4], were also examined, as it was thought that they *might* be of varietal significance.

Of these characters, however, the only important one, so far as observations went, is length of lateral, as this is the most easily ascertained, and the other characters are dependent on that. With increase in length of lateral there is an increase in length of secondary laterals, and in length of internode; of the last two the rate of increase is not the same for both, that for the internodes being greater than that for the secondary laterals, so that a short lateral is densely flowered while a long one is lax. A typical long lateral may be contrasted with a typical short lateral as:

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[1] Those laterals below the breast-wire are, together with the lower leaves, stripped off before "hop-washing" commences.

[2] By first internode is here meant that between the first and second pairs of bracteoles. When the bracteoles were not truly opposite, but were more or less alternate, the node was taken as being midway between the two bracteoles representing an opposite pair.

[3] Sooner or later along the lateral the bracteoles towards the distal end are represented merely by scales.

[4] By the "stipular inflorescences" is meant those short laterals at the base of each main lateral and growing apparently in the axils of the stipules.

Long lateral (see Fig. 1).	*Short lateral* (see Fig. 2).
Very lax	Very dense
Length 4 to 5 feet	Length 9 to 15 inches
Longest secondary lateral 6–9 in.	3-4 in.
First internode 5–7 in.	1–2 in.
Bracteoles nearly all opposite	Mostly alternate.

The development of a lateral is so easily modified by external factors that there is usually considerable variation in the same individual, and though some plants evince a tendency to produce long laterals, others short ones, no one plant has hitherto been met with possessing laterals consistently of the long or of the short type as defined above. Perhaps the best example in the garden of a ♂ hop with the tendency to produce long laterals is one growing in hills G 27, 319, 320. Those at the lower level[1] are very lax, measure 1 ft. 9 in. to 5 ft., and bear secondary laterals up to 8 in. in length; at the higher level they are less lax and from 1 ft. 6 in. to 3 ft. 6 in. in length with secondary laterals up to 9 in.

A male hop with very short dense laterals is J 36, which during the past three years has borne laterals 9 in. to 1 ft. 3 in. long at the lower level and 7 to 12 inches at the higher, with secondary laterals 3 to 4 inches long; in this particular case they are exceptionally short, and this may be due to the fact that the plant is still rather young, though it is to be remarked that the laterals were, on the whole, a little shorter in 1914 than in 1913, which seems to indicate that the plant has already reached a stage of development when length of lateral is no longer modified by the age of the plant. A typical plant bearing short laterals may be considered as one producing laterals which are in general about 15 inches long, but occasionally reaching a maximum of 2 ft. 6 in. for the lower level and 2 ft. for the higher.

The factors determining length of lateral are probably very complex but the more evident of these may here be noted. Young seedlings and hills raised from cuttings that have recently been planted have invariably short dense laterals; as the vigour of the plant increases so these inflorescences increase in length year by year until a certain maximum is attained. The laterals are usually somewhat pendant, but when they reach a suitable support they often assume the twining habit characteristic of the stem and in consequence are stimulated to increased growth. Again, should a bine lose its growing point by careless training the upper laterals grow out at an increased rate and simulate bines. Such anomalies are to be ignored when determining length of lateral.

[1] See page 178.

The pedicels and the outer surface of the perianth lobes of those plants with red bines are tinged with red, the laterals of those plants thus appearing of a reddish-green before the flowers open. An interesting feature in connection with the Oregon ♂ hops is that the laterals are more "open," *i.e.* the flowers not so crowded together as in the English form. On the re-appearance of this character in 1913 it was more closely studied and the difference was seen to be in the greater rigidity of the flowering branches of the Oregon plants, so that they stand out from the axis (primary, secondary, etc., as the case may be) bearing them more nearly at right angles than in the English forms, where the branches are more or less drooping unless supported (see Fig. 3). In the former again the pedicel is usually strongly curved at right angles immediately beneath the flower, and there is a tendency for the secondary laterals to bear the tertiary branches unilaterally. Whether the condition that obtains in the Oregons is the more advantageous for the dissemination of the pollen is not certain, but it would seem to permit of a more *uniform* distribution of the pollen than would be the case where the flowers are crowded together in a pendant tassel.

With regard to the stipular laterals there is little to be remarked. In some cases they are long and tapering (a maximum of 12 inches has been observed), in others short and rounded, while sometimes they are all suppressed with the exception of a few towards the top of the plant.

The Stipules.

These are interpetiolar; every leaf is provided with two, each of which is partially fused with the one on the same side belonging to the other leaf at that node, thus at each node there appear to be two bifid stipules alternating with the two leaves. The stipules may be upright exposing the lower surface, spreading, or recurved exposing the upper surface. In the English forms the stipules are upright to spreading, usually almost at right angles to the axis of the bine, while in the Oregon hops, although a few may be spreading the tendency is for the stipules to be so recurved that the outer surface of the tips becomes adpressed to the stem below the node.

The Flower.

Perianth. The five lobes of the perianth vary in size from $\frac{1}{8} \times \frac{1}{10}$ to $\frac{1}{10} \times \frac{1}{20}$ inch practically on every plant, but the former dimensions are the more frequently met with in the English forms, the latter in the Oregon males.

On the outer surface of the lobes are glands, which are smaller and less conspicuous than those of the leaves. The number (per each lobe) is by no means constant even on the lobes of the same flower and for any one plant is usually from 0 to 8 per lobe. Occasionally one meets with extreme types like No. 279 in which the number is frequently 15, a lobe with glands absent altogether being of very rare occurrence, and No. 83 of which the lobes are usually eglandular but may have 1 to 3 glands, no more than 3 per perianth lobe having been observed in this plant. In the case of the Oregon males the number is generally well above the average and may reach 20.

Glands of the anthers. Along the dorsal (outer) furrow of each anther may be found a number of comparatively large glands, arranged in a single row when they are few but frequently biseriate when more than 10 are present. In the English forms the number is from 0 to 8 (usually 2 to 4); 9 or 10 are rarely found and 11 were counted on one occasion only. With the exception of the last all anthers with more than 10 glands have been found only on the Oregon plants and on seedlings with an Oregon plant as one of the parents. In the Oregon males themselves the number of glands per anther varies from 4 to 18 (usually about 10) and the glands are often biseriate in the furrow. Among the English plants none has been conspicuous in producing very few glands except perhaps A 15 and I 31, in each of which the number is usually 0 to 2 and no more than 4 have been seen on an anther of either plant.

The glands of the disc. The disc from which the stamens arise is also often glandular, but this point was not noticed until too late in the season of 1912 for full observations to be made that year[1] though it was given attention during the two succeeding years. These glands are minute and individually invisible to the naked eye, therefore easily overlooked except when numerous. Here again the number often varies considerably in the same plant, from 0 to 15 being a range of variation frequently encountered at a hill; yet extreme types are to be recognized. Thus one plant (C 3) has borne glands from 10 to 25 in number, another (I 17) 5 to 25, and in these they are quite conspicuous when observed by means of a hand-lens; in the Oregon ♂ hop on the other hand no disc-glands have yet been discovered and apparently are not developed in that plant. This feature of the Oregon male is of interest since in the case of other glandiferous organs, viz. leaves, anthers, and perianth lobes, these plants produce more numerous glands than the average plant of English origin.

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[1] Afterwards it was found that these glands are figured in Braungart's *Der Hopfen*, p. 206.

The Oregon Male Hops.

A comparison of the characters of the four hills of male hops obtained from Oregon leads to the conclusion that they are all of the same variety, and that this variety differs from the English male hops in certain well-marked features. The differences drawn up in tabular form will serve to emphasize this point.

	Oregon ♂ hop	English ♂ hop
Leaves	Almost flat	Usually more or less wrinkled and margin recurved
Colour of leaves	Light green	Variable
Lobes of leaf	5–11 with tendency for terminal lobe to be trilobed	3–5 (very rarely 7), terminal lobe entire
Apex of lobes	Acuminate	Obtuse
Length of terminal lobe	About ¾ the whole length of the lamina	⅔ (usually less) length of lamina
Breadth of terminal lobe	About ½ length of lobe	About equal to length of lobe
Base of terminal lobe	Narrow; about ½ width of lobe	Broader, more than ½ width of lobe
Furrow of petiole	Shallow	Usually deep
Stipules	Reflexed	Upright to spreading
Glands of anthers	Usually more than 10	Usually less than 10, often absent
Disc of flower	Eglandular	Usually glandular
Perianth lobes	Usually conspicuously glandular	Glands usually 0 or few and scattered
Inflorescence	Rigid and "open"	Less rigid, drooping

The leaf-glands of the Oregon ♂ hop are generally more numerous than in the English males, though among the latter occasionally a plant is met with possessing leaves as glandular as in the former. The differences, particularly those concerned with the stipules and the shape of the leaf, are so marked that it appears advisable to consider the Oregon form to possess specific rank distinct from *H. lupulus* L.; this point will be found discussed elsewhere[1].

Type Characters.

The observations recorded in the foregoing pages show that although in the English male hops there are certain characters which, within fairly narrow limits, are constant for the individual plant, there is between the extremes in each case a series of intervening forms—a series which is so near being perfect, that it is perhaps impossible to formulate a scheme of classification that will include and yet distinguish all the forms. At present it seems best to define the characters that may be taken as *extreme types* in each series and to employ these as standards in comparing the various forms that already exist or new forms that may arise as hybrids or as mutations. It is proposed therefore to select as type characters the two extremes in each of the four series, where (as shown in this paper) variation is considerable when applied to a large number of plants (such as were available at Wye) but confined within comparatively narrow limits when applied to the individual.

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[1] Salmon, E. S. and Wormald, H.: "*Humulus Americanus* Nuttall," *Journal of Botany*, May, 1915, pp. 132–135.

I. *Time of Flowering*[1].

(*a*) *Early*—about July 1st, and may be during last week in June or first week in July.
e.g. No. 294 [= 295] June 30th–July 4th. Average July 2nd.
F 3 June 28th–July 5th. Average July 2nd.

(*b*) *Late*—about August 1st, and may be during last week in July or first week in August.
e.g. E 16 [= 44; 48] July 23rd–Aug. 6th. Average July 28th.
I 31 Aug. 2nd–Aug. 9th. Average Aug. 5th.

For use in pollination it will also be necessary to select intermediate types that will be in flower when the midseason ♀ hops are in burr.

e.g. C 3 July 9th–12th. Average July 10th; early midseason.
H 22 [= 26] July 16th–25th. Average July 20th; late midseason.

II. *Colour of Bine.*

(*a*) *Green*, *e.g.* E 16 [= 44; 48].
(*b*) *Red*, *e.g.* H 22 [= 26].

III. *Length of Laterals.*

(*a*) *Long and lax*, reaching a length of 4 or 5 feet.
e.g. E 16 [44; 48] and G 27 [319; 320].

(*b*) *Short and dense*, maximum length 2 feet 6 inches, but usually shorter than that.
e.g. J 36; 67; H 10.

IV. *Glands on the Leaves.*

(*a*) *Numerous*—average over 60 for ¼ in. circle taken as described on p. 183; minimum greater than 40.
e.g. H 22 [= 26] with an average of 73.
168 [= 167; 169] with an average of 82.

(*b*) *Few*—average under 30; maximum less than 40.
e.g. 67 with an average of 23.
296 [= 297] with an average of 28.

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[1] *Vide* p. 178 for definition of "Time of Flowering" as used in this paper.

Such characters as roughness of bine, colour of leaves, recurving and wrinkling of leaves, length of internodes, etc., are perhaps not sufficiently distinctive for types to be defined, but they may be useful in distinguishing varieties which show a constant feature in one or more of these characters.

The variation in the glandulation of the disc, anthers, and perianth-lobes of the flowers serves chiefly to distinguish the Oregon and the English plants, though it is possible that forms may arise among the latter which will resemble the former in one or more of these characters; some of the English plants, as already shown, approach the Oregons in their relative high number of glands on the perianth-lobes. It also provides a basis of observation when investigating the transmission of characters to the hybrid offspring obtained by crossing the two forms. Whether a male hop which produces a large number of glands will transmit that character to its progeny (male and female) is a question that only future experiments will decide, but it is presumable that such a plant would be more valuable in that direction than one in which the factors necessary for gland production are but feebly developed. Should breeding experiments prove that this is the case the value of observation concerning this character in the males will be enhanced.

In accordance with the above scheme of *type-characters* (limited to four pairs of such characters) it is conceivable that we may have 16 *type-varieties*, each of which will possess 4 of those characters, one of each pair. Of the male hops hitherto examined only one conforms to this idea of type-varieties, and but one hill of this hop (No. 67) has been available, so that the constancy of its characters is somewhat uncertain, though, since it was planted out in the garden in 1909, the features characteristic of the mature plant were probably established when observations commenced in 1912. During the three seasons 1912–13–14 it has shown the following type-characters:

I *a*, early flowering (June 26th–July 14th; av. July 5th).

II *b*, red bine.

III *b*, short laterals (8 in. to 2 ft. 6 in.).

IV *b*, few glands on leaf (16–31; av. 23).

A few plants possess three type-characters, others two, *e.g.* E 16 [= 44; 48] is late in flowering (I *b*), has a green bine (II *a*), and long laterals (III *a*); its average number of glands is 41, so that in this respect it is an intermediate form.

The extent of variation in the cultivated ♂ hop from the original ♂ of the wild *Humulus lupulus* has not been determined, as it is highly probable that many of the "wild" hop-plants now growing in this country, particularly in Kent and the other hop-growing counties, are seedlings from the varieties cultivated. Mr Salmon, however, has received from Professor P. A. Saccardo, who assures us that it has been obtained from the true wild hop in Italy, seed of *H. lupulus* labelled "Vittorio, ad sepes, *omnino sponte*, Oct. 1913. In Italia *Humulus* non colitur[1]." Plants are being raised from this seed, and when mature will be brought under observation.

Scope and Aim of Work on Male Hops.

There are two distinct lines of investigation in connection with the male hop. The first of these is concerned with the selection of those forms which are most suitable for planting commercially among the ♀ plants in hop-gardens. Obviously such plants must be selected primarily for their time of flowering, for unless this coincides with that of the ♀ plants among which they are growing they are useless, and it is evident too that vigour (including suitability for different soils) must be considered. The selection of those cuttings of male hops sent out from Wye College to hop-growers for the purpose of providing a supply of pollen is based on these two characters.

The second line of investigation is to determine how ♂ hops can be used to the best advantage in breeding operations, that is to say, how far a judicious selection of these plants for use in crossings will result in a higher percentage of commercially valuable seedling ♀ hops. This will involve (1) a comparison of the vegetative characters of the ♂ plants with those of the ♀ plants to determine which of these characters are associated with what are considered as good qualities in the ♀ plant, (2) a selection of those ♂ plants showing one or more of the characters which are considered to be useful, in order that such plants may be employed in crossing, (3) careful observation of the progeny obtained from the crosses to determine the potency of the ♂ parent in transmitting desirable qualities to the ♀ offspring.

The present paper shows that male hops exhibit definite variation in several directions and that selection for one or more characters is quite feasible.

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[1] See, however, *Journ. of Bot* May, 1915, p. 135

Whether any pair of characters (as, for example, the type-characters enumerated above) are allelomorphic pairs is a problem the solution of which has not yet been attempted.

To the student of genetics the hop-plant is not an ideal subject, because of the comparatively long interval[1] that must inevitably elapse between the inception of two successive generations, so that it may be many years before any definite results can be obtained. Hop-growing is, however, such an important industry in this country that we cannot afford to neglect the application of modern methods in plant-breeding and selection to the hop-plant, in an endeavour to obtain new varieties superior in quality or in vigour to those already grown, particularly as this plant is now receiving the attention of scientific experts both on the continent and in America.

In conclusion I desire to express my thanks to Mr E. S. Salmon, F.L.S., of the Research Department, South-Eastern Agricultural College, whose advice during the progress of the work has been most valuable, and also to Mr F. Summers, M.Sc., Botany School, Cambridge University, who kindly made extracts from works in the library of the University.

EXPLANATION OF PLATE IV.

FIG. 1. A typical *long, lax* lateral with its subtending bract.

FIG. 2. Portion of a hop-bine bearing *short, dense* laterals.

FIG. 3. Above is shown a *rigid, open* lateral of an Oregon ♂ hop; below is the drooping type of lateral of the English forms.

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[1] At Wye no seedling has been known to flower during its first season, although Dr Johs. Schmidt finds that at Copenhagen his seedlings frequently come into flower the first year (see *Comptes-rendus des travaux du Laboratoire de Carlsberg*, 11me Vol. 1915, p 170); even during the second season the plants do not attain to their full vigour and it is evident that certain characters at any rate are not constant at that age.

(*Received May 5th*, 1915.)

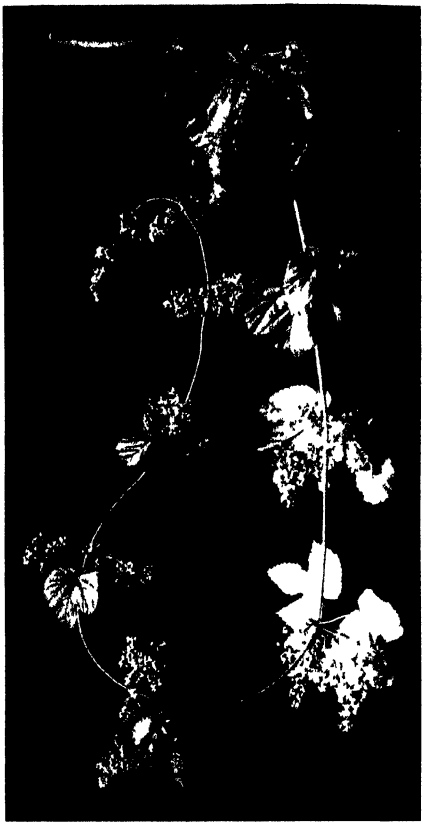


Fig 1

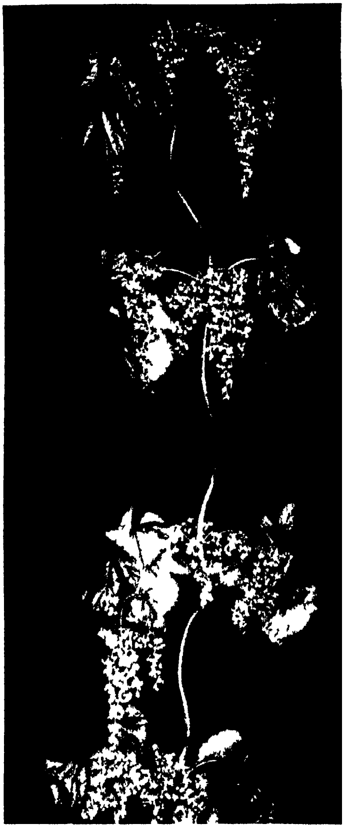
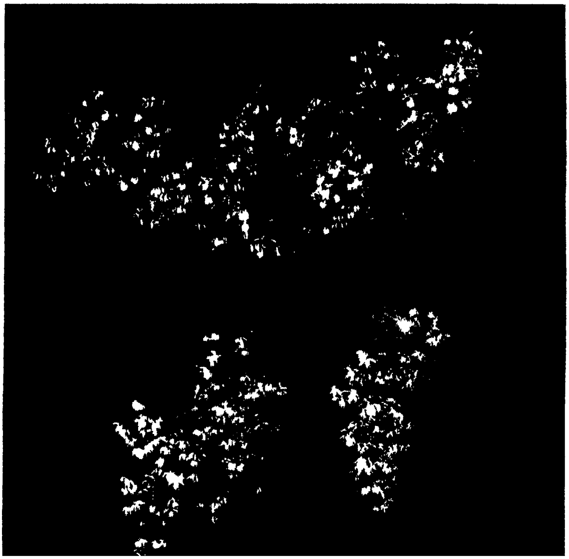


Fig. 2.



THE INFLUENCE ON CROP AND SOIL OF MANURES APPLIED TO PERMANENT MEADOW.

By CHARLES CROWTHER and ARTHUR G. RUSTON.

(*Department of Agriculture, The University, Leeds.*)

The observations which form the basis of the present communication have been made in connection with a comparative test of different systems of manuring meadow land which has been carried on continuously and uniformly since 1899 at the Manor Farm, Garforth[1]. Precisely similar tests were made for several years at five other centres in the West Riding of Yorkshire, the tests being continued at three centres for eight years. At Garforth the plots still continue to be manured in accordance with the original scheme.

This scheme was designed to test the following points:

(*a*) The effects of an annual dressing of dung.

(*b*) The effects of a dressing of dung every two years.

(*c*) The effects of alternate annual dressings of dung and various artificial manures.

(*d*) The effects of "complete" and "incomplete" mixtures of artificial manures.

(*e*) The comparative effects of nitrate of soda and sulphate of ammonia.

The detailed scheme of manuring is given in Table I.

The soil of these plots at Garforth is a dry, light loam, poor in lime, resting upon sandstone in the Middle Coal Measures series. The plots are each $\frac{1}{20}$th acre in extent. Dung is applied usually in March, but occasionally earlier. Superphosphate and kainit are applied usually in March, sulphate of ammonia in March to early May, and nitrate of soda in late April or early May.

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[1] Experimental Farm of the University of Leeds and the Yorkshire Council for Agricultural Education.

The plots are mown when the majority are in full flower,—usually early July,—and the produce weighed as hay. The aftermath is grazed by lambs receiving cake, etc. This fact needs to be kept in mind in considering the results, as do also the relatively light annual rainfall of 20·25 inches, and the poverty of the soil in calcium carbonate.

TABLE I.

Plot No.	Manure per acre 1899 and alternate years subsequently	Manure per acre 1900 and alternate years subsequently
1	No manure	
2	Dung, 10 tons *	
3	Dung, 10 tons *	No manure
4	”	Nitrate of soda, 1½ cwt.
5	”	Nitrate of soda, 1½ cwt. Superphosphate, 26 %, 2 cwts.
6	”	Nitrate of soda, 1½ cwt. Superphosphate, 2 cwts. Kainit, 3 cwts
7	Nitrate of soda, 1½ cwt. Superphosphate, 2 cwts. Kainit, 3 cwts.	
8	Sulphate of ammonia, 130 lb. † Superphosphate, 2 cwts. Kainit, 3 cwts.	
9	Nitrate of soda, 1½ cwt. Superphosphate, 2 cwts.	
10	Sulphate of ammonia, 130 lb. † Superphosphate, 2 cwts.	
11	Nitrate of soda, 1½ cwt.	
12	Sulphate of ammonia, 130 lb. †	
13	No manure	

* For year 1913 and subsequently, only 6 tons.

† Supplying nitrogen equal in amount to that applied on Plot 7.

Effects upon Yield of Hay.

It is not proposed to discuss here the detailed records of the hay crops obtained on the different plots. The summary given in Table II will perhaps suffice to indicate the general character of the effects produced by the manuring.

TABLE II. *Yield of Hay* (*per acre*). 16 *Years* (1899–1914).

Plot No.	Maximum	Minimum	Average	Increase over average of unmanured plots	
	cwts.	cwts.	cwts.	cwts. =	per cent.
1	40¼	18	27·9 ± 1·1 } *	—	—
13	37	14¾	25·7 ± 1·1 } *		
2	70	37½	50·6 ± 1·6	23·8 ± 1·9	89 ± 7
3	57¼	28¼	44·9 ± 1·3	18·1 ± 1·6	68 ± 6
4	66½	38¼	49·4 ± 1·4	22·6 ± 1·7	84 ± 6
5	60¼	37¼	48·8 ± 1·1	22·0 ± 1·4	82 ± 5
6	67¾	36¾	50·5 ± 1·5	23·7 ± 1·8	88 ± 7
7	55	29	41·2 ± 1·3	14·4 ± 1·6	54 ± 6
8	51¾	20¼	35·9 ± 1·3	9·1 ± 1·6	34 ± 6
9	55	28¼	39·0 ± 1·3	12·2 ± 1·6	45 ± 6
10	47¼	26¾	34·2 ± 1·1	7·4 ± 1·4	28 ± 5
11	49	25¼	34·6 ± 1·2	7·8 ± 1·5	29 ± 5½
12	47¼	20¾	29·0 ± 1·2	2·2 ± 1·5	8 ± 5½

* Average yield on unmanured plots = 26·8 cwts. ± 1·0.

The main features of the average yields may be summarised as follows:

(1) With one exception (Plot 12—sulphate of ammonia alone) the manured plots all show substantial increases over the unmanured plots.

(2) The plots which have received dung (Plots 2–6)—not excepting even Plot 3, which in alternate years was entirely unmanured—show much more substantial increases than those which have received only "artificial" manures (Plots 7 12).

(3) Nitrate of soda has proved markedly superior to sulphate of ammonia (*cf.* Plots 7 and 8; 9 and 10; 11 and 12).

(4) The use of sulphate of ammonia alone (Plot 12) has proved very unsatisfactory.

(5) Within the limits of error of experiment alternate annual dressings of dung and nitrate of soda (Plot 4) have given as high yields as any other treatment (*cf.* with Plots 2, 5, 6).

(6) The indications as to the manurial needs of the soil with respect to supplies of nitrogen, phosphate and potash are as follows:

	Increased yields on	
	Nitrate plots (7, 9, 11) per cent.	Ammonia plots (8, 10, 12) per cent.
Effect of supplying nitrogen [Plots 11, 12 compared with 1, 13]	29±5½	8±5½
Effect of supplying phosphate [Plots 9, 10 compared with 11, 12]	16±8	20±7½
Effect of supplying potash [Plots 7, 8 compared with 9, 10]	9±8½	6±7½

The results indicate a marked response to nitrogenous manuring in the form of nitrate of soda, a much less sharply defined response to phosphate, and no measurable response to potash. The reasons for the poor response to nitrogen when applied in the form of ammonium salts will be dealt with later.

In considering the records of experiments of this character the averages can give only a partial indication of the real effects of the differences in treatment of the various plots. It is desirable to study also the changes step by step throughout the whole period of the experiment. It is not our intention, however, in the present communication to enter into detailed discussion of the yearly records. We would merely indicate that the yearly records show little falling-off as yet in the productivity of the unmanured plots, whilst the plots receiving dung seem to have reached practically the limits of their productive powers, giving on the average a crop which is roughly double that on the unmanured plots. This is the case even on Plot 3 (dunged and unmanured in alternate years). The records of this plot show a steady improvement in condition with the result that in the later years (prior to the reduction in the dressing of dung in 1913) the yields obtained from this plot were almost equal to those obtained from any other plot of the dung series, as the following summary shows:

Average Yields (per acre) for the Five Years 1909-13.

Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
cwts.	cwts.	cwts.	cwts.	cwts.
50·8±3·0	47·3±1·8	52·0±2·6	51·5±2·2	53·2±2·8

In 1914 the yield from Plot 3 fell considerably below that from the other "dung plots," but it is, of course, too early yet to say whether this is purely a seasonal fluctuation or is to be attributed to the reduction in the dressing of dung in 1913 from 10 tons to 6 tons per acre.

Of the plots receiving "artificials" only (Plots 7-12), the nitrate plots (7, 9, 11) have, in the main, kept up the relative standard of fertility established at the start, whilst the ammonia plots (8, 10, 12) all show signs of losing ground. This is notably the case with Plot 12 (ammonia alone) which in six years of the series has given yields no greater than those obtained from the adjoining unmanured Plot 13.

Effects upon Character of Herbage.

It is now a familiar observation that the continued manuring of grass-land may produce marked changes in the character of the herbage[1]. That this has been the case in the experiments here under review is obvious to the eye and is clearly defined in the results of botanical surveys of the plots that have been made from time to time.

The first of these was made by J. N. Cameron[2] in May and June 1906, the method adopted being to take four specimen turves from each plot, tease out the different plants, sort out into species and weigh after removal of the roots. The weights were then calculated as percentages of the total herbage. The results were also checked by a rougher examination of the herbage of much larger areas of the plots. The great disadvantage of the method lay in the extreme difficulty of getting turves really representative of the whole plot, since the grasses tend to grow in patches.

A further botanical analysis of some of the plots (Nos. 1, 2, 3, 6, 7, 9, 11, 12) was made in 1909 by J. R. Bond[3], the samples this time being taken from the cut grass lying in the swaths immediately after mowing (July 22). Portions were taken from various parts of each swath, and then the whole combined sample from each plot, weighing several pounds, was dried, separated into its constituent species, and these weighed.

In order to complete the records the plots not dealt with by Bond were sampled by one of us in July 1911 and dealt with on precisely the same lines. Further analyses of some of the plots were made in 1914.

In comparing the results of the three sets of observations it must be borne in mind that the quantitative distribution of the various species that compose the flora of a meadow may vary greatly from year to year and also at different periods of one and the same season[1]. This would account for the fact that Cameron, taking samples in May and June, found much lower proportions of the late flowering grasses, such as bent, than were found in the later analyses based upon samples taken in July. Apart from differences of this character the various observations show substantial agreement. The results of the later analyses are summarised in Table III.

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[1] *Cf.* Stapledon, This *Journal*, VI. 499.

[2] Results embodied in thesis submitted at Final B.Sc. Examination, 1907.

[3] Results embodied in thesis submitted at Final B.Sc. Examination, 1910. See also "Guide to Experiments at Manor Farm, Garforth," 1914, pp. 6, 7.

TABLE III.

Name	Number of Plot												
	1	2	3	4*	5*	6	7	8*	9	10*	11	12	13*
	%	%	%	%	%	%	%	%	%	%	%	%	%
1. *Lolium perenne*	·1	1·2	1·5	1·0	2·0	·7	·9	1·2	1·2	1·1	1·8	1·4	·6
2. *Alopecurus pratensis* ...	2 8	16·1	5·3	6·1	1·6	9·0	2·0	·1	3·8	—	1·1	·4	·1
3. *Dactylis glomerata*	3·5	14·9	12·0	5 8	10·8	19·9	29·7	4·8	26·8	5·9	27·8	12·3	3·3
4. *Avena flavescens*	8·2	4·3	34 3	20·4	24·2	20·4	31·9	7·2	13·4	1·5	7·0	·7	1·6
5. *Poa trivialis*	—	3·6	1·9	·1	—	2·0	·1	·6	·2	·5	—	—	—
6. *Poa pratensis*	·2	—	·1	·1	—	·1	·2	·4	·1	·4	·2	·5	—
7. *Festuca ovina et duriuscula*	1·9	·1	1·3	1·9	·5	·9	4·6	3·0	2·6	5·1	7·4	1·1	1·4
8. *Arrhenatherum avenaceum*	1·1	3·1	3·8	1·4	1·6	9·8	·2	·2	8·4	—	·1	·5	—
9. *Anthoxanthum odoratum*.	3·3	—	·2	·9	·2	·02	·2	14 2	·8	8·5	1·1	4·5	5·7
10. *Agrostis vulgaris*	53·4	·2	1·4	3·7	1·9	1·1	12·6	58·8	9·0	71·4	23·4	57·3	74·0
11. *Bromus mollis*	—	13·6	1·9	14·3	32·8	2·7	·3	·2	·7	·1	·4	·1	·1
12. *Holcus lanatus*	2·4	·6	3·2	6·2	·7	·8	2·4	5·0	2·2	2·1	1 7	1·1	1·6
13 *Rumex acetosa*	15·4	16·1	24 3	34 4	21·1	18·6	9·1	1·4	20·7	2·3	14 7	10·2	4·1
14. *Heracleum sphondylium* .	·5	10·3	·5	1·1	1·6	10·2	·8	·3	2·1	—	2 0	·5	—
15. *Anthriscus sylvestris*	—	10·2	3·5	-	1·2	·3	—	·2	- -	—	-	—	—
16. *Bunium flexuosum*	2 2	·1	·4	—	·1	·4	·9	·7	1·8	·2	2 1	1 1	5 4
17. *Luzula campestris*	·2	—	·01	—	—	—	·03	·4	·01	·2	3	·5	·7
18. Various other weeds ...	·03	·05	·2	·1	·04	·1	—	1·2	·01	·1	·1	·1	·8
19. *Lathyrus pratensis*	—	—	·1	1·0	—	—	·3	—	—	—	- -	- -	—
20. *Trifolium pratense*......	—	—	—	1·0	—	—	—	—	—	·03	·01	—	·05
21. Undetermined	4·8	5·6	4·1	·5	·7	3·0	3·8	·1	6·2	·6	8·8	7·7	·6

* Analysed in 1911 by Ruston. All other plots analysed in 1909 by Bond.

It will be noted that the dominant grass on the unmanured land (Plots 1 and 13) is *Agrostis vulgaris*, but that this has been very largely suppressed on the dunged plots (2–6). It persists to a considerable extent on the plots manured with artificials only (7–12), being much less pronounced on the nitrate plots (7, 9, 11) than on the ammonia plots (8, 10, 12). ***Rumex acetosa*** is prominent throughout and is increased rather than diminished on the dunged plots. It is least pronounced on the plots receiving "complete" artificials. Other well-defined changes effected by the application of dung are the encouragement of *Dactylis glomerata*, *Alopecurus pratensis*, *Avena flavescens*, *Bromus mollis* and the large umbelliferous weeds *Heracleum sphondylium* and *Anthriscus sylvestris*.

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[1] *Cf.* Armstrong, This *Journal*, 1907, ii. 299.

On the "artificials" plots (7–12) *Dactylis glomerata* and *Agrostis vulgaris* are conspicuous grasses, whilst *Rumex acetosa* is a prominent weed; *Alopecurus pratensis* and *Bromus mollis* have made no headway, whilst *Avena flavescens* is prominent only on Plot 7 (nitrate, superphosphate, kainit) and Plot 9 (nitrate, superphosphate); the umbelliferous weeds are practically absent.

On comparing the nitrate plots (7, 9, 11) with the ammonia plots (8, 10, 12) it will be seen that the use of nitrate has apparently encouraged, or at least sustained, *Alopecurus pratensis*, *Dactylis glomerata*, *Avena flavescens*, and *Rumex acetosa* and has repressed *Agrostis vulgaris*. It is interesting to note that the use of ammonium sulphate along with superphosphate (Plots 8 and 10) has very greatly checked the growth of *Rumex*—a weed whose presence in appreciable amount is commonly regarded as indicative of acidity or "sourness" in the soil.

The general character of the herbage may perhaps be conveniently summarised as follows, using a purely arbitrary classification of the grasses:

Plot No.	Good grasses (Nos. 1–8, Table III)	Inferior grasses (Nos. 9–12, Table III)	Leguminous plants (Nos. 19 and 20, Table III)	Miscellaneous weeds (Nos. 13–18, Table III)
	per cent.	per cent.	per cent.	per cent.
1	17·8	59·1	—	18·3
2	43·3 (36·4 †)	14·3 (21·4)	— (3·0)	36·8 (34·1)
3	60·2	6·8	·1	28·9
4 *	37·7	24·2	1·1	35·6
5 *	40·9	35·0	—	24·0
6	62·7	4·6	—	29·5
7	69·6 (75·3)	15·5 (14·1)	·3 (2·0)	10·8 (8·6)
8 *	31·7 (11·7)	63·9 (78·4)	— (0·4)	4·1 (8·0)
9	56·6	12·7	—	24·5
10 *	22·9	73·6	·03	2·8
11	45·4	26·7	·01	19·2
12	17·0 (1·8)	62·9 (87·4)	— (—)	12·5 (10·6)
13 *	12·9 (3·4 ‡)	75·7 (90·2 ‡)	·05 (0·1 ‡)	11·1 (4·7 ‡)

* See note to Table III.

† Figures in brackets refer to samples taken in 1914.

‡ Averages of Plots 1 and 13 in 1914 crop.

The preponderance of "inferior grasses" on the unmanured plots (1 and 13) and on the plots dressed with sulphate of ammonia (8, 10, 12) is brought out very clearly in this summary.

As indicated in the table a comparison is possible in the case of five plots of the composition of the herbage in two different years, viz., 1909 (or 1911 for Plots 8 and 13) and 1914.

The unmanured plots (1 and 13) show a marked increase in the inferior grasses, which the detailed results (not given here) show to be due to a great development of bent (*Agrostis vulgaris*).

The annually dunged plot (Plot 2) shows no great alteration in the proportions of the different groups, but there have been changes within the groups. Thus in 1914 the proportion of cocksfoot (*Dactylis glomerata*) was much lower, and of golden oat grass (*Avena flavescens*) much higher than in 1909. Of the inferior grasses Yorkshire fog (*Holcus lanatus*) had increased considerably.

On the plot receiving annual dressings of nitrate, superphosphate, and kainit (Plot 7) the general distribution was much the same in the two crops but cocksfoot was lowered and rye-grass increased. Bent was appreciably more abundant as were the umbelliferous weeds.

On the two plots receiving sulphate of ammonia (Plots 8 and 12) the composition of the two years' crops was greatly different, the later crop containing a much smaller proportion of the better grasses, notably cocksfoot, golden oat grass and the fescues, whilst the inferior grasses, notably bent and Yorkshire fog, were correspondingly increased. In agreement with the indications of Table III, sorrel (*Rumex acetosa*) was considerably reduced on these plots.

A further point of interest brought out in the earlier work of Cameron is the variation in the proportion of dead grass leaves that persist about the bases of the plants. Estimations made by him of the proportion of dead grasses to total herbage on each plot resulted as follows:

Plot	%	Plot	%
1	21·6	8	29·7
2	4·6	9	8·9
3	9·9	10	28·0
4	8 5	11	9 2
5	11·7	12	33·5
6	11·6	13	22 8
7	13 1		

Attention may be specially directed to the high proportions on the unmanured plots and especially on the "ammonia plots" (8, 10, 12). The point is one of considerable interest to which we shall return later.

Chemical Composition and Estimated Nutritive Value of the Hay Crop.

In order to arrive at a complete record of the effects of the different manurings upon the hay crop it is necessary to know not only the yields of hay but also the relative nutritive value of the produce from each plot. This latter can be assessed either directly by feeding experiments or indirectly from the chemical composition of the hays. In the case of the Garforth experiment the size of plot used precluded the application of the direct method, so that recourse could only be had to chemical analysis. Various observers[1] have shown how unreliable are the indications of present methods of analysis as a guide to the relative feeding

TABLE IV. *Chemical Composition of Hay.*

Plot No.	Crude protein	True protein	Digestible true protein *	Crude fibre	Ash	Ether extract	N-free extractives	Moisture in hay †
	Per cent. in dry matter							%
				1909 crop				
1	8·1	—	–	28·9	5·3	57·7		
2	9·3	—	—	32·0	6·5	52·2		
3	8·7	—	—	31·3	5·8	54·2		
7	9·5	—	-	26·4	6·2	57·9		
9	9·8	—	—	29·2	6·2	54·8		
11	10·4	—	—	28·7	6·4	54·5		
12	11·6	—	—	27·8	5·8	54·8		
				1911 Crop				
1	8·1	6·8	5·1	29·0	6·3	3·1	53·5	7·9
2	9·0	7·0	5·3	36·2	8·6	3·4	42·8	7·1
3	8·7	7·2	5·4	33·3	7·3	3·2	47·5	9·3
4	9·0	7·5	5·7	32·6	8·2	2·7	47·5	9·4
5	8·8	8·0	4·9	30·6	7·2	3·2	50·2	8·9
6	8·6	7·2	5·0	30·5	7·6	3·7	49·6	9·4
7	8·3	7·8	6·1	24·8	7·1	3·0	56·8	6·6
8	8·7	6·7	4·6	29·7	6·7	2·0	52·9	9·1
9	9·5	7·8	6·2	28·2	7·5	4·3	50·5	10·9
10	9·6	7·4	5·7	28·3	6·2	2·6	53·3	9·0
11	10·7	8·2	6·1	26·3	6·8	3·1	53·1	9·8
12	11·1	7·7	5·6	28·2	5·8	2·2	52·7	10·7
13	8·4	6·9	4·8	28·5	6·5	2·5	54·1	10·1
				1914 Crop				
1 and 13	9·3	7·7	4·4	26·1	6·5	3·5	54·6	8·6
2	10·4	7·8	3·6	31·0	7·2	3·4	48·0	9·4
7	9·5	8·6	4·5	24·9	6·7	3·9	55·0	8·4
8	9·6	8·3	4·0	28·1	6·5	3·2	52·6	8·5
12	11·8	9·3	4·6	27·0	6·4	3·0	51·8	9·7

* Laboratory determinations with acid solution of pepsin. † At time of analysis.

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[1] *e.g.* Hall and Russell, This *Journal*, 1912, IV. 339.

values of different pastures, but it is not unreasonable to expect that in the case, such as the present, of adjoining plots on a small area of one and the same field, the indications will be more reliable.

The produce of some of the plots was sampled and analysed by Bond in 1909. Two years later (1911) the crops on all the plots were again sampled and analysed by us. On five plots further sampling and analysis took place in 1914. In every instance the samples were taken at the time of cutting, and dried entirely under cover. The results of the analyses are summarised in Table IV.

The results of the three series of analyses show fair agreement in general, the following points being common to each:

(1) With but one exception (1911 Crop, Plot 7) the proportion of crude protein is greater on the manured than on the unmanured plots (1 and 13).

(2) The enrichment in crude protein is greatest on those plots where nitrogenous manure alone was applied (Plots 11 and 12).

(3) The proportion of crude protein is lower on the plots manured with nitrate of soda (7, 9, 11) than on the corresponding ammonia plots (8, 10, 12).

(4) The differences above referred to are due rather to the non-protein than to the true protein fraction of the crude protein.

(5) The proportion of crude fibre is markedly higher on the dunged plots than on the rest.

(6) The lowest proportion of crude fibre and the highest proportion of nitrogen-free extractives ("soluble carbohydrates") are found on the plot receiving a "complete" mixture of artificials including nitrogen in the form of nitrate of soda (Plot 7).

(7) The proportion of ash in the hay grown with nitrate of soda (7, 9, 11) is higher than that in the hay from the corresponding ammonia plots (8, 10, 12).

In interpreting the results both here and elsewhere it must be borne in mind that the hay on the dung plots was dead ripe at the time of cutting. This reveals itself more particularly in the increase of crude fibre. Late cutting and the presence of a relatively large proportion of tall, coarse grasses and weeds all tend to give a hay containing a high proportion of crude fibre. These are the conditions which obtained on the dunged plots.

In assessing the nutritive value of the produce from each plot it is necessary to take into account the digestibility of the material.

This we can only arrive at in the present instance by indirect means since direct digestion trials with animals could not be carried out. The procedure adopted for our present purpose is that followed by us in a previous paper[1].

The digestible protein, as arrived at in the laboratory, is given in Table IV. The "amides" are assumed to be completely digestible. The sum of digestible "carbohydrates" and fibre is taken as equal to the total "carbohydrates" (Henneberg and Stohmann's Rule). The "ether extract" is assumed to be one-half digestible. The results are summarised in Table V.

TABLE V. *Estimated Digestible Constituents of Hay.*

Plot No.	Per cent. in dry matter				Total yield per acre			
	True protein	"Amides" (non-protein N × 6¼)	Ether extract	"Carbohydrate" and fibre	True protein	"Amides"	Ether extract	"Carbohydrate and fibre
	%	%	%	%	lb.	lb.	lb.	lb.
				1911 Crop				
1	5·6	1·3	1·5	53·5	145	35	40	1393
2	5·7	2·0	1·7	42·8	248	84	74	1846
3	5·9	1·6	1·6	47·5	285	78	78	2287
4	6·2	1·5	1·3	47·5	293	72	63	2218
5	5·3	0·8	1·6	50·2	255	39	77	2393
6	5·5	1·4	1·8	49·6	264	67	90	2400
7	6·6	0·5	1·5	56·8	233	18	53	2021
8	5·0	2·0	1·0	52·9	139	55	28	1500
9	7·0	1·7	2·1	50·5	217	49	64	1504
10	6·2	2·2	1·3	53·3	171	29	35	1449
11	6·7	2·5	1·5	53·1	180	67	29	1395
12	6·2	3·4	1·1	52·7	130	70	23	1093
13	5·4	1·4	1·2	54·1	111	28	26	1101
				1914 Crop				
1 and 13	4·4	1·5	1·6	49·9	81	28	29	915
2	3·6	2·3	1·5	43·5	151	97	63	1827
7	4·5	0·6	1·8	50·4	146	18	58	1637
8	4·0	2·2	1·4	48·2	91	50	32	1093
12	4·6	2·3	1·3	46·7	111	55	31	1124

From the data in Table V we have calculated the "starch equivalents" of each crop, proceeding upon the lines laid down by

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[1] This *Journal*, 1912, IV. 305.

Kellner[1], as outlined in our earlier paper[2]. The results are embodied in Table VI.

TABLE VI. *Estimated Starch Equivalents of Crops from Different Plots.*

Plot No.	Per 100 lb. dry matter		Total per acre		Relative values of crops (unmanured = 100)		
						In terms of starch equivalents	
	For maintenance	For production	For maintenance	For production	From weights of hay alone	For maintenance	For production
	lb. (1)	lb. (2)	lb. (3)	lb. (4)	(5)	(6)	(7)
				1911 Crop			
1 and 13	58	39	1334	897	100	100	100
2	51	27	2117	1121	180	159	125
3	54	33	2552	1559	206	191	174
4	53	33	2439	1518	200	183	169
5	55	37	2572	1730	203	193	193
6	55	36	2613	1710	207	196	191
7	64	47	2173	1598	148	163	178
8	56	39	1526	1063	118	114	119
9	57	39	1696	1160	131	127	129
10	58	40	1595	1100	120	120	123
11	59	42	1564	1113	115	117	124
12	57	39	1183	810	90	89	90
				1914 Crop			
1 and 13	59	42	1082	770	100	100	100
2	52	33	2183	1386	227	202	180
7	60	44	1949	1430	176	180	186
8	57	39	1293	885	123	119	115
12	56	38	1348	915	130	125	119

It will be seen that when the estimated nutritive value of the hay is taken into account (cols. 6, 7) the dung plots (2–6) do not show quite so great an advantage over the others as when judged by weight of hay alone (col. 5).

In the two years to which the data refer the crop of highest feeding value per 100 lb. (Table VI, cols. 1, 2) was evidently that from Plot 7 ("complete" artificials including nitrate of soda). When both yield and quality are taken into account (cols. 3, 4) the best results (1911 crop) are shown by Plots 5 and 6 which received dung and artificials alternately (dung for 1911 crop). Of the five plots sampled in 1914, the "continuous dung" plot (Plot 2) compares unfavourably with Plot 7 in feeding value per acre. On examining further the records of this plot (dung annually) it is noted that in each year although the crop on this plot was much heavier than that on Plot 7, it was generally so inferior in feeding value that the actual value per acre was distinctly less.

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[1] *Scientific Feeding of Farm Animals*, pp. 82–93.

[2] *loc. cit.* p. 311.

Removal of Manurial Ingredients by Crop.

In addition to the ordinary analysis of the hays from the plots in 1911, determinations were made of the phosphoric acid, potash and lime present in them, with the results summarised in Table VII.

TABLE VII. *Manurial Ingredients present in Hay* (1911).

Plot No.	Nitrogen	Phosphoric acid (P_2O_5)	Potash (K_2O)	Lime (CaO)	Ratio CaO : P_2O_5
	%	%	%	%	
1	1·20	0·36	0·62	0·43 *	1·16 : 1 *
2	1·34	·57	2·25	·53	0·93 : 1
3	1·27	·51	2·18	·59	1·16 : 1
4	1·31	·67	2·26	·67	1 : 1
5	1·28	·56	1·87	·66	1·18 : 1
6	1·25	·51	1·54	·64	1·26 : 1
7	1·25	·54	1·28	·65	1·20 : 1
8	1·26	·50	1·50	·50	1 : 1
9	1·35	·52	0·63	·73	1·41 : 1
10	1·40	·94	0·76	·66	0·70 : 1
11	1·54	·41	1·12	·46	1·12 : 1
12	1·58	·36	·75	·44	1·22 : 1
13	1·21	·39	·68	—	—

* Average of Plots 1 and 13.

The data show clearly that, with perhaps the exception of the potash, the composition of the hay with regard to manurial ingredients is a very uncertain guide to the actual manurial treatment. The phosphoric acid figures show far less variation than the potash, whilst the nitrogen varied least of all. The proportions of lime are uniformly low in accordance with the poverty of the soil in this ingredient. More especially are the ratios of lime to phosphoric acid much below those taken as normal for meadow hay (2–2½ : 1). The point is of interest as having a possible bearing upon the feeding value of the grass[1].

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[1] *Cf.* Ingle, This *Journal*, 1910, III. 22.

Influence upon Chemical Composition of Soil.

Analyses were also made in 1911 of the soils of the different plots and it is of interest to compare the results obtained with the soils with those given above for the crops. The essential data are summarised in Table VIII[1].

TABLE VIII. *"Available" Plant Food in Soils.*

Plot No.	"Available" phosphoric acid	"Available" potash	Calcium carbonate
	%	%	%
1 and 13	0·012	0·007	0·06
2	·028	·051	·14
3	·019	·025	·16
4	·023	·043	·21
5	·016	·036	·11
6	·015	·043	·15
7	·018	·027	·14
8	·019	·025	·08
9	·013	·010	·16
10	·015	·022	·06
11	·013	·008	·11
12	·010	·015	·01

It will be found on comparison that there is a fair degree of correlation between Tables VII and VIII, as, for example, in the accumulation of potash on the dunged plots and its scarcity on Plots 1 and 9–13. It is further of interest to note the depletion of the supplies of available potash on Plots 9 and 11 which receive nitrate of soda as compared with the corresponding ammonia plots (10, 12).

There is little sign of correlation with regard to phosphoric acid. The lowest proportion is recorded on Plot 12 (ammonium salts alone), a fact which is in accord with the known power of ammonium salts of facilitating the taking up of phosphates by plants.

The general lowness of the supplies of calcium carbonate has been referred to already. It will be observed how the use of sulphate of ammonia on Plots 8, 10 and 12 has accentuated this poverty.

The indications of soil analysis with regard to the calcium carbonate were borne out by the results of determinations of the "lime-fixing" powers of the soils of the plots, made by shaking up separate equal

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[1] The chemical composition of the soils was more exhaustively studied but it is not proposed to deal with the results here.

TABLE IX. *Showing Volume of Acid (N/10) required to neutralise "unfixed" Lime remaining after Treatment of Soil with increasing Proportions of Lime.*

Lime	Plot 2	Plot 7	Plot 8	Plot 12
%	c.c.	c.c.	c.c.	c.c.
·25	1·4	2·5	1·2	0·5
·5	2·8	5·0	2·6	0·8
·75	5·2	7·6	4·1	2·0
1·0	8·4	10·3	5·0	2·5
1·25	9·8	15·2	6·8	4·0
1·5	11·0	15·4	8·6	5·2
1·75	17·6	21·6	10	7·3
2·0	35·3	40·1	20·2	10

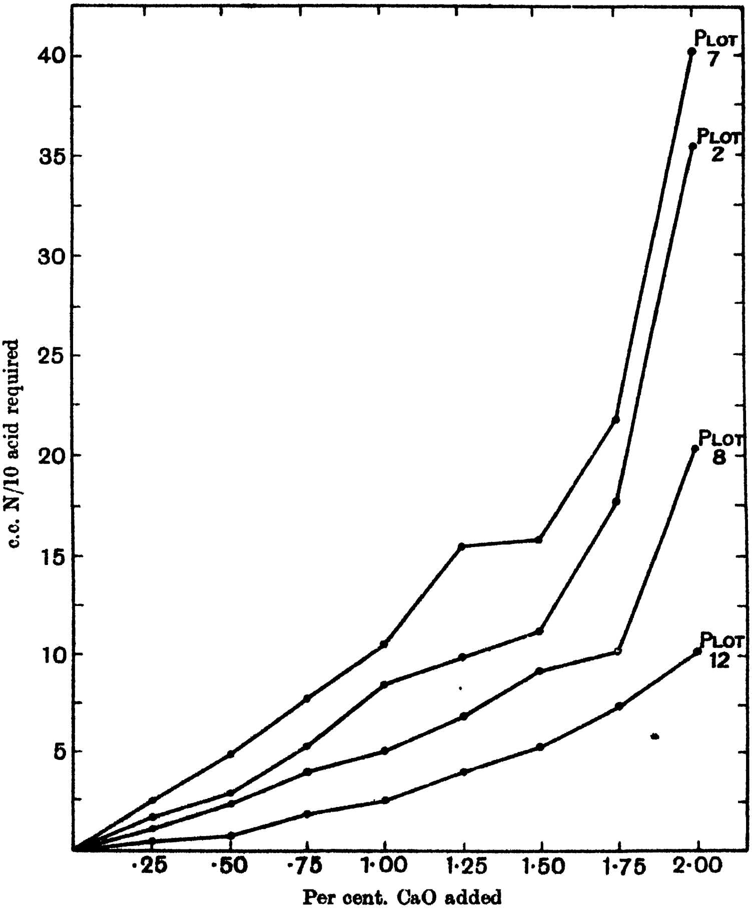

Fig. 1.

portions (100 gms.) of soil with water and varying amounts of lime, and determining the lime left free after twenty-four hours.

It was found that the soil of Plot 12 "fixed" practically the whole of the lime applied even when the proportion was as high as two per cent. of the soil used, whilst even in the most favourable case the soil fixed practically 1·5 per cent. of its weight of lime before any appreciable surplus of free lime remained. The data for a few plots are given in Table IX (*cf.* also Fig. 1).

Influence upon Bacterial Activities in Soil.

The part played by soil bacteria in determining fertility, more particularly by regulating the supply of available plant food, is now common knowledge and need not be enlarged upon. In this connection interest centres mainly round the supply of nitrogen, which is presented by the bacteria first in the form of ammonia and then, if the conditions are favourable, the latter is further converted to nitrates. Under normal conditions this latter change ("nitrification") goes on more rapidly than ammonia production and hence the amount of ammonia present in the soil at a given time is usually exceedingly small. In the case of ordinary arable soils investigated by Russell[1] the ammonia amounted to only 1-2 parts per million of soil, rising to 3-4 parts in the case of rich dunged soils and garden soils. Much higher proportions were found, however, in the soils of the Garforth hay plots here under review, as may be seen in Table X.

TABLE X. *Nitrogen as Ammonia and Nitrate in Soils* (1911).

Plot No.	Total nitrogen	Nitrogen present in form of ammonia	Nitrogen present in form of nitrate
	Parts per million	Parts per million	Parts per million
1 and 13	1640	4·0	3·2
2	2490	15·1	7·6
3	2300	7·3	11·3
4	2200	7·6	10·5
5	1910	9·8	8·7
6	1940	11·6	7·4
7	1840	15·6	6·1
8	1870	16·4	1·6
9	1760	12·8	4·3
10	1940	18·1	1·2
11	2100	12·6	5·6
12	1860	7·1	0·2

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[1] Russell, This *Journal*, 1910, III. 233.

The proportions of nitrogen (total) in the soils are much what one would expect from the manuring, the dung plots (2–6) having accumulated considerable reserves.

The ammonia results can be explained by assuming that the conditions for nitrification were much more unfavourable than in the soils examined by Russell. This is highly probable in view of the low proportions of calcium carbonate in the soils. The case of Plot 12 (ammonium salts alone) is specially interesting, the relatively low proportion of ammonia in this soil as compared with Plots 8 and 10 indicating that on this plot the conditions are becoming unfavourable even for production of ammonia (see later).

The nitrates present in the soils at the time of analysis were in no case high, and indeed on the ammonia plots were hardly measurable.

Further evidence of the low bacterial activity on some of the plots is furnished by the presence (*cf.* p. 204) round the bases of the grasses of a decided mat of undecayed vegetable matter, this being notably the case on Plots 8, 10, and 12. That this acts as a handicap to the grasses by withholding water from their roots was illustrated by samples of some of the soils taken to a depth of 9 inches on September 17, 1912. The determinations of moisture gave the following results:

Plot	Moisture
12	16·7 per cent.
10	17·8 ,,
8	19·2 ,,
7	21·7 ,,
3	24·7 ,,
2	28·6 ,,

The difference of 12 per cent. between Plots 2 and 12 will represent roughly a difference of 120 tons of water in the supply per acre, equivalent to a rainfall of 1¼ inch. Similar observations in a neighbouring pasture with limed and unlimed plots—the unlimed plot having a thick "mat"—lead to the same conclusions and indicate further the beneficial effect of the liming upon bacterial activity. These results are summarised below:

Plot	Date of test	Moisture in soil
Unlimed	Nov. 9, 1911	8·1 per cent.
Limed	,, ,,	21·5 ,,
Unlimed	Oct. 30, 1913	11·1 ,,
Limed	,, ,,	18·7 ,,

The indirect evidence of marked differences in bacterial activity on the different plots was fully borne out by direct determinations, made in 1911, the results of which are summarised in Table XI.

TABLE XI. *Bacterial Activity in Soils.*

Plot No.	Total no. of bacteria* per ·0001 gm. soil	Relative activity on different plots (unmanured = 100)			Relative activity of Catalase ferment‖ (unmanured = 100)	Undecayed grasses (per cent. of total herbage) (Cameron) *cf.* p. 213	Moisture content of soils on Sept. 17, 1912
		Ammonia-producing bacteria†	Nitrate-producing bacteria‡	Nitrogen-fixing bacteria§			
1 and 13	42	100	100	100	100	22·2	21·5 %
2	192	107	383	236	233	4·6	28·6
3	108	104	323	168	186	9·9	24·7
4	112	103	246	119	177	8·5	25·0
5	117	101	283	100	163	11·7	25·3
6	128	103	217	100	167	11·6	24·2
7	82	99	263	81	87	13·1	21·7
8	62	89	79	75	77	29·7	19·2
9	45	99	142	75	99	8·9	22·8
10	16	88	31	49	91	28·0	17·9
11	32	95	94	63	80	9·2	20·5
12	4	82	13	37	61	33·5	16·7

* Colonies on Agar incubated 3 days at 38° C.
† After 3 days' incubation (5 gms. soil).
‡ ,, 21 ,, ,, at 28° C. (5 gms. soil).
§ ,, 7 ,, ,, (5 gms. soil).
‖ ,, 3 hours' ,, at 28° C. (5 gms. soil).

It will be observed that, with the exception of the production of nitrates on Plot 7, the number and activity of the organisms were relatively greater on the dunged plots than on the plots which were either unmanured or received only "artificials." Further that bacterial activity of all kinds was much greater on the nitrate plots (7, 9. 11) than on the corresponding ammonia plots (8, 10, 12). The adverse conditions for bacterial life on Plot 12 are strikingly exemplified. The soils which showed the lowest bacterial activity (8, 10, 12) showed also the lowest moisture-content and carried the biggest proportion of dead undecayed grasses (*cf.* p. 213).

The differences would appear to be least pronounced with regard to the ammonia-producing organisms, but further tests showed that the data recorded in the table scarcely give a fair indication of the real differences in ammonia-producing power of the different plots. The data given in the table were arrived at by incubating 5 gms. of soil for three days at 28° C. with 80 c.c. of a nutrient solution containing 10 grms. of peptone per litre. In view of the small differences in ammonia production thus obtained, further estimations were made with varying amounts of peptone and different periods of incubation. The general character of the results is illustrated by Fig. 2. It will be seen that

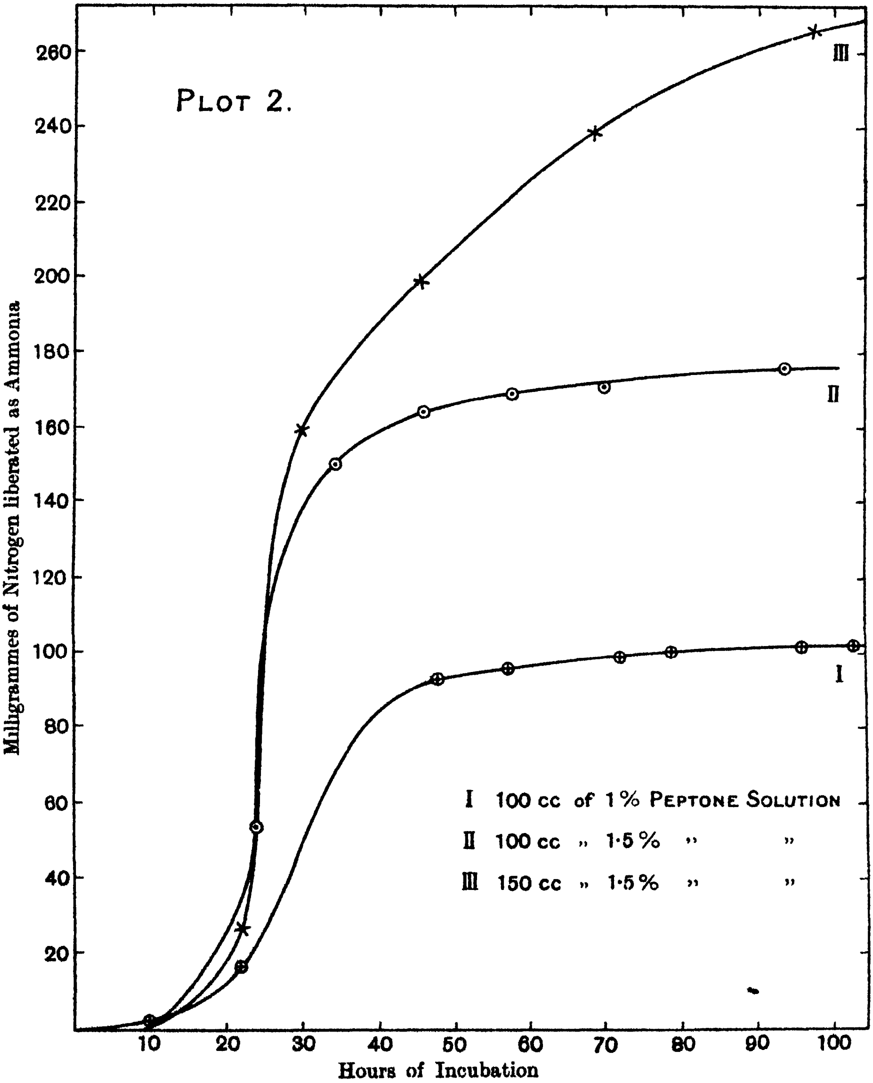

Fig. 2.

the amount of ammonia produced is greatly influenced by the supply of peptone, but that in all cases the rate of production falls greatly after about 50–60 hours' incubation. This latter conclusion was in the main borne out by similar tests with other soils, but deviations from the general rule were not lacking.

On repeating now the comparative tests with the different soils, using 100 c.c. of the 1 per cent. peptone solution and estimating the ammonia present after different periods of incubation, much more marked differences were found than those recorded in Table XI. The following comparison of the best and worst plots will serve as an illustration (see also Fig. 3). For convenience of comparison the amount of ammonia produced by Plot 12 after 48 hours' incubation is taken as 100. The figures in brackets give the amounts for Plot 2, taking the amount for Plot 12 for the same period as 100.

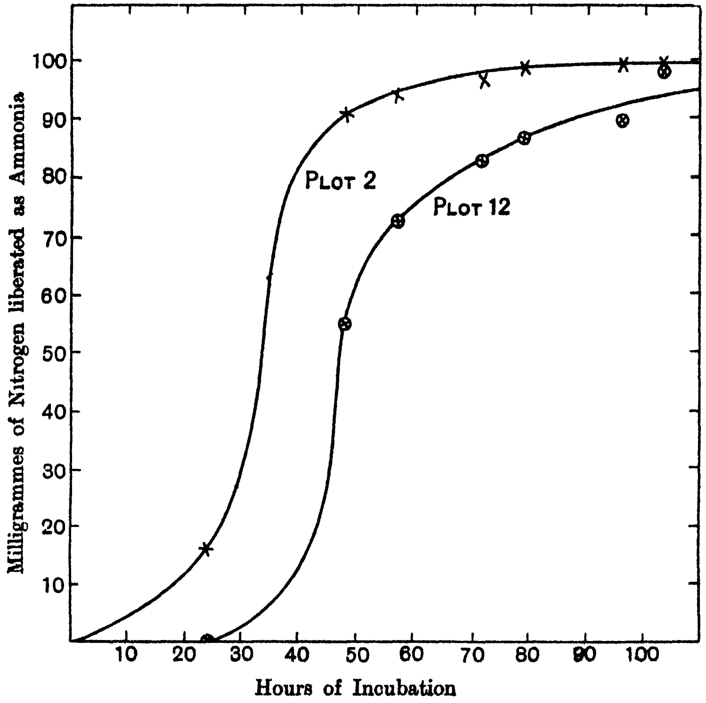

Fig. 3.

Relative Amounts of Ammonia produced during

	24 hrs.	48 hrs.	57 hrs.	72 hrs.	79 hrs.	96 hrs.	103 hrs.
Plot 2	29	164	171 (130)	175 (117)	179 (114)	180 (111)	180 (101)
Plot 12	Nil	100	132	150	157	162	179

Thus at the end of 48 hours the ammonia output on Plot 2 was 64 per cent. more than on Plot 12, whilst by the end of the third day (72 hours) the superiority had been reduced to 17 per cent., and after 103 hours the two plots showed practically equal records. Had we been able to establish conditions such as prevail on the plots, where the ammonia is largely removed as it is produced, the superiority shown by Plot 2 in the earlier stages would doubtless have been maintained throughout. It will be noted further that whereas the maximum output in the case of the soil from Plot 2 was practically reached in 48 hours, more than twice this period was required in the case of Plot 12.

Generally speaking, the ammonia-production was decidedly more active on the dunged plots (Plots 2–6) than on the "artificials" plots (Plots 7–12). This is exemplified by the following data obtained by incubating 5-gram portions of the different soils with 100 c.c. of 1·5 per cent. peptone solution.

Milligrams of Ammonia produced during

	9 hrs.	24 hrs.	34 hrs.	46 hrs.	58 hrs.	70 hrs.	94 hrs.
Plot 2	1·5	53·9	150·5	164·6	168·8	170·5	175·7
„ 7	·8	14·2	100·3	129·2	130·0	147·4	165·4
„ 8	Nil	6·8	39·5	120·3	112·8	130·3	163·4

The superiority of the dunged plot (Plot 2) throughout the first half of the incubation period is seen to be very marked, whilst Plot 7 (complete artificials including nitrate of soda) shows to advantage in the earlier stages in comparison with Plot 8 (complete artificials, including sulphate of ammonia). This latter difference was reproduced also in the comparison of the other nitrate plots (9, 11) with the corresponding ammonia plots (10, 12). The poverty in respect of calcium carbonate of the soil throughout the plots is doubtless largely responsible for this relatively unfavourable biological condition of the ammonia plots.

SUMMARY.

The experiments reviewed in the preceding pages have been carried out on a light loam soil very poor in lime, in a district of medium rainfall (20–25 inches).

The chief conclusions drawn from the results are as follows:

1. Although the heaviest crops have been obtained with an annual application of dung, they are little heavier, and more costly to obtain, than the crops obtained with a biennial application of dung, especially

if in the alternate year a light dressing of "artificials" including nitrate of soda be given.

2. A complete mixture of "artificials," including nitrate of soda, has given good average crops, but not equal to those given by a biennial application of dung.

3. For the soil and other conditions of Garforth nitrate of soda is distinctly better for the hay crop than sulphate of ammonia. This is doubtless largely associated with the poverty of the soil in calcium carbonate.

4. The different manurings have effected marked and characteristic changes in the botanical composition of the herbage. In particular the continued use of ammonium salts has led to serious deterioration.

5. There are now differences also in the chemical composition of the herbage, which probably represent substantial differences in feeding value. For equal weights, the hay grown with dung appears to have a lower feeding value than that grown with a good mixture of "artificials."

6. The composition of the ash of the hay does not reflect the character of the manuring, except with regard to potash.

7. Substantial changes in the power of the soil to supply plant food have taken place as a result of the manuring. The most marked effect is the removal of carbonate of lime by the prolonged use of ammonium salts.

8. The effects—direct and indirect—of the manuring upon the soil have led to marked differences in bacterial activity. In some cases the reduction in biological activity is so great that dead grasses accumulate and form a mat on the soil so thick that the penetration of water to the lower layers is seriously impeded.

9. In comparing the ammonia-producing powers of soils it is desirable that estimations of ammonia produced be made at relatively short intervals, say every twelve hours, since the *rate* of production in the earlier stages is a more characteristic index of the activity of the soil than the *total output* after prolonged incubation.

(*Received April 16th,* 1915.)

THE THEORY OF WETTING, AND THE DETERMINATION OF THE WETTING POWER OF DIPPING AND SPRAYING FLUIDS CONTAINING A SOAP BASIS.

By W. F. COOPER, B.A. (Cantab.), F.C.S.,
and W. H. NUTTALL, F.I.C., F.C.S.
(*From the Cooper Laboratory for Economic Research, Watford.*)

The dipping of sheep and cattle, as a means of eradicating 'scab,' lice, ticks, etc., and the diseases which it is now known the latter may transmit, has met with such success, that compulsory dipping is now in vogue in most pastoral countries. Where compulsory dipping obtains, there must of necessity be some system of the standardisation of dips. In Queensland and South Africa, the respective Governments issue official formulae from which the stockbreeder can prepare his own dipping fluid. Only such proprietary dips, as are duly recognised by the Government, may be employed. In the United States, the regulations for the sale of proprietary dips are still more stringent. The quantity of active substance, usually sodium arsenite, nicotine or cresylic acid, is defined within very narrow limits. Further, no proprietary dip is now recognised, unless the manufacturer can furnish a 'Field Tester,' by means of which the stockbreeder can himself determine, in a simple and fairly trustworthy manner, the percentage of active constituent in his bath.

The underlying idea, upon which all these regulations are based, is that the principal factor, which determines the efficacy of a dipping fluid, is the quantity of *toxic* substance present. It has been shown, however, in experience derived from field experiments and routine dippings, that this assumption is unwarranted. There is another factor of almost equal importance, *viz.*, the capacity of the dipping fluid to *wet* the greasy hide or fleece. A plain aqueous solution of sodium arsenite, containing 1 part of arsenious oxide in 600 parts of water, may be quite ineffective as a dip, whilst a solution of the same concentration, but containing in addition a small percentage of soap and oil to increase the wetting power, proves to be quite satisfactory. The fact that the Queensland official dip contains soap as a basis may be quoted as an indication that the importance of the wetting power of dipping fluids is recognised in actual practice.

The difficulty of determining the wetting power of a solution has, however, precluded this point being considered in the comparison of dips in the laboratory.

What has been said of dipping fluids, refers with equal force to horticultural spraying fluids—a high percentage of *toxic* substance is no criterion of the efficiency. The supreme importance of the spray fluid having a *high wetting power* is gradually becoming more and more recognised; though not generally by Government authorities. In the United States, compulsory spraying is rapidly becoming the rule, and the standardisation of spraying fluids is its natural consequence. This standardisation merely considers the percentage of the *toxic* agent, and entirely disregards the equally important point of the wetting power.

A simple laboratory test for the determination of the relative wetting powers of different preparations is in urgent demand, and various methods of comparison have been suggested from time to time. Generally speaking, these suggestions have not been based upon an exact knowledge of the principles underlying the process of wetting, and, in consequence, the results obtained by the use of such methods are of little value.

The generally accepted test is the determination of the surface tension of the preparation. Thus, for example, Brünnich and Smith (1914, p. 83) state that 'the wetting power of any liquid, or its property to form a uniform film upon a greasy surface, depends primarily upon its surface tension,' and they attempt to compare the wetting power of various liquids by measuring their surface tension by means of a stalagmometer. Vermorel and Dantony (1910, pp. 1144–5) distinguish between the *static* and *dynamic* surface tensions of soap solutions, and claim that the value of the static tension, as opposed to the true (or dynamic), affords a satisfactory criterion of the wetting power. They point out that two solutions of sodium oleate of concentration 5 % and 0·1 % respectively, give the same capillary rise and wet equally well; nevertheless their true surface tensions differ very considerably. In a later paper, however (1912, pp. 1300–1), they state that 'the surface tension of a liquid is not sufficient to determine its wetting power....

The wetting power manifests itself very differently, according to the nature or the state of the substance to be wetted....With regard to plants, the wetting power of some solutions appears to depend less upon the surface tension than upon the surface viscosity, as defined by Plateau.'

Lefroy (1915, pp. 291–3), in a paper dealing with the precise mode of action of *contact* poisons in insecticides, emphasises the importance of the wetting power of a spray-wash, and also touches upon the theory of wetting. He points out, that the capacity of a liquid to wet a solid surface is determined by the relative values of three distinct tensions: *viz.*, those of the Wash/Solid, Wash/Air and Air/Solid. He states that the condition for the wash to wet the solid is that the tension Wash/Solid must be less than the sum of the other two, *viz.*, Wash/Air and Air/Solid[1]. He points out that, as the tension Air/Solid remains constant, and the tension Wash/Solid is indeterminate, the tension Wash/Air is the only one to be considered, and the lower this tension is, so the more readily will the wash wet. Thus Lefroy's method of comparing the wetting power is nothing more than a determination of the surface tensions of the respective washes.

Other methods of comparing the wetting power of solutions have been based upon a comparison of the amount of liquid which adheres to a solid object of standard shape and size, which is dipped into the liquid and then carefully withdrawn. Naturally, the nature of the solid employed for the purpose is of considerable importance, and of those, the use of which has been suggested, mention may be made of the elytra of *Blatta Periplaneta americana*, strips of celluloid and of grease paper.

Most of the cattle dips in actual use contain a certain proportion of emulsified oil. It has been claimed that the presence of this emulsified oil increases the wetting power of the dip, and further, that the finer the state of this emulsion, the more marked is this effect. It has been urged, that the wetting power of such emulsified dips may be measured by a comparison of the fineness of the emulsified oil particles, and attempts have been made to compare the latter by observing the relative capacity of the dips to give a permanent foam.

A knowledge of the percentage of soap in any preparation gives no indication whatever as to its wetting power, so that the latter cannot be determined by means of a chemical analysis. Vermorel and Dantony (1911, pp. 972–4) have shown that the actual mode of preparation of a fungicide has a marked influence upon its subsequent wetting power. For instance, two liquors, identical in their content of copper sulphate and sodium carbonate, but *prepared under diverse conditions*, required in the one case eight kilos of soap, in the other case only two kilos of soap to produce an equal wetting power.

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[1] See footnote, p. 228.

Before we are in a position to appraise at their real value, these methods for determining the wetting power of liquids, it will be necessary to consider in some detail the various factors upon which the process of wetting depends. Though these factors were described in the main by Quincke (1877, p. 149) at an early date, and have since been referred to in various textbooks, yet they are so little understood by the ordinary chemist or biologist, that one may be excused for reiterating them here.

A drop of rain, falling freely through the air, assumes a spherical form, and behaves as if it were covered with a tightly stretched elastic film. The spherical form is the result of the pressure exerted by this surface film, and the film pressure itself is usually termed *surface tension.*

Now consider the case of a drop of oil, suspended in dilute alcohol of the same density as the oil (Plateau's experiment). The drop of oil also assumes a spherical form, as if bounded by a tightly stretching film. Obviously, the two cases are perfectly analogous, and the shape of the drop of oil is the result of a tension exerted at the interface of the oil and dilute alcohol. This tension is the surface tension of the oil to the dilute alcohol, but for the sake of distinction is usually known as the *interfacial tension.* Such a tension will exist at the interface of any two immiscible liquids, though in some cases it is known to be extremely small.

Consider now the surface of a solid. The idea of a stretched film exerting a distinct tension at the surface of a solid is not so readily conceived as in the case of a liquid. Nor can the existence of a solid surface tension be easily demonstrated experimentally. Nevertheless certain theoretical considerations place it beyond doubt that such a tension does exist at the surface of a solid[1].

Further, in the case of a liquid in contact with a solid, judging from analogy, we should expect an interfacial tension to exist between the liquid and the solid; and this is known actually to be the case.

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[1] Surface tension may be regarded as surface energy per unit area, and it follows therefore that part of the energy of a solid body may be regarded as proportional to its surface, and that in this sense the body has a surface tension, this tension being measured by the energy per unit area of the surface.

We are now in a better position to understand the main factors upon which the wetting of a solid surface depends. Let us imagine that a drop of liquid has been placed upon the surface of a solid. Before we can decide whether it will retain its form, or whether it will spread out and cover the surface as a continuous film, *i.e.* wet the surface, consideration must be given to the relative values of three distinct tensions[1].

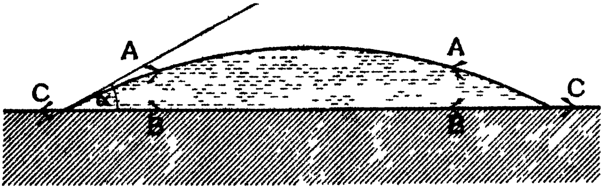

Fig. 1.

There is the surface tension of the liquid, which we will designate T_1; the surface tension of the solid T_2; and the interfacial tension of the liquid to the solid T_{12}. The surface tension of the liquid T_1 will be exerted in the direction of the arrows at A (Fig. 1), and will tend to maintain the spherical form of the drop. Similarly, the interfacial tension T_{12} will be exerted in the direction of the arrows at B, and this tension will also tend to roll up and maintain the drop in its spherical form. The surface tension T_2, however, will have the opposite effect; it will be exerted in the direction of the arrows at C, and will strive to draw out the drop into a more and more lenticular form, and the angle of contact (a) of the drop becomes smaller and smaller, until finally the latter forms a continuous film over the surface of the solid. For this to happen, it is not difficult to see that the surface tension of the solid T_2 must exceed the sum of the surface tension of the liquid T_1 and the interfacial tension T_{12},

i.e. $$T_2 > T_1 + T_{12}.$$

Since two of these tensions, *viz.*, the surface tension of the solid T_2, and the interfacial tension T_{12}, cannot be determined experimentally, it is impossible to apply this equation to a simple practical test. Röntgen (1878, pp. 324–8), however, has succeeded, by indirect means, in demonstrating its validity for the system Rubber/Water.

From the above equation, it is evident that for a liquid to possess a high wetting power, it shall have a low surface tension (T_1) and also

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[1] If the drop of liquid is sufficiently small, the effect of gravity need not be considered.

a low interfacial tension (T_{12}). Further, it is evident from the consideration of Fig. 1, that the effect of reducing the interfacial tension T_{12} will be to cause the liquid to spread over the surface of the solid, even if the surface tension T_1 is fairly high; or in other words, it is the interfacial tension T_{12}, rather than the surface tension of the liquid T_1, which is the determining factor in wetting power.

The process of wetting, however, is not so simple as would appear from a consideration of the above equation. There are at least two other factors which must be taken into account, which, under certain conditions, may cause the above relationship to fail entirely.

The first of these disturbing factors is concerned with the solvent properties of the wetting liquid. It almost always happens in actual practice that the surface to be wetted is already coated with a layer of some greasy or waxy secretion. Since wetting depends partly upon the interfacial tension of the liquid to the solid, actual contact between the two is essential. It is necessary therefore that the wetting liquid should have a certain solvent action on the grease or wax, with which the solid may be coated. This solvent power need not be very pronounced, provided that the interfacial tension of the liquid is small, for, provided the surface of the solid is reached in any one place, the greasy or waxy layer will be displaced by the wetting liquid, because its interfacial tension to the solid will be lower than that of the grease or wax to the solid. If, on the other hand, the grease or wax is completely insoluble in the liquid, there can be no actual contact between the solid and the liquid, and therefore no wetting. Thus, for example, it is probable that the reason why mercury fails to wet the surface of so many solids is not entirely due to its high surface tension, but to the fact that it is incapable of dissolving the surface layer of moist air which adheres to the surfaces of most solids (Freundlich, 1909, p. 176).

The second factor, liable to vitiate conclusions drawn from the equation conditioning wetting, is concerned with the phenomenon known as *surface concentration* (capillary adsorption). If, with increasing concentration, an aqueous solution of a substance decreases the surface tension, it will be found that the solute tends to aggregate in the surface layers of the solution (Milner, 1907, p. 96). Saponin, proteins and various other substances possess this property to a marked degree. Thus, for example, Lewis (1908, p. 513) has calculated that with a 0·25 % solution of sodium glycocholate, there is an extremely thin surface layer, the degree of concentration of which is about 160 times that of the bulk. The result of this surface concentration is the peculiar superficial viscosity or rigidity, first observed by Plateau (1873, pp. 261–96). In the case of saponin, this surface viscosity or rigidity may be readily demonstrated. If a bubble is blown with a 1 % solution of saponin, the bubble film will be found to be imperfectly elastic, and quite different from a soap film. If air is withdrawn while the orifice of the pipe is held in a vertical plane, so as to disturb the symmetry of the bubble, the shrinking film assumes a crinkled appearance, until finally there results a vertical semi-solid cylinder with almost opaque walls.

Vermorel and Dantony (1912, pp. 1300–1) were the first to point out that a liquid, exhibiting surface viscosity to a marked degree, had in consequence a high wetting power. Solutions of saponin have marked wetting properties, although the surface tension of such solutions is comparatively high. Thus, a 1 % solution of saponin is capable of wetting a glass plate, coated with paraffin wax, although a 5 % solution of soap fails to do so. The wetting power of solutions of saponin, gelatine, etc., seems to depend largely upon their capacity to form liquid planes, the high superficial viscosity of which prevents rupture and running together to form drops.

The phenomenon of surface concentration is, however, of interest to us from another point of view. It has long been observed that the apparent surface tension (static) of soap solutions is practically identical for all concentrations between 10 % and 0·1 % (Marangoni, 1871, p. 342, footnote). More recent investigations have, however, shown that this statement is only true, if the surface tension determination has been carried out on an *old* surface. Rayleigh (1890, p. 285) succeeded in showing that, if the surface tension of a 0·25 % solution of sodium oleate is measured within 1/400 of a second after the formation of the surface, the surface tension (dynamic) approximates closely to that of water. This initial tension, however, quickly falls until the ordinary value for the surface tension is reached. Rayleigh's results have been repeatedly confirmed by other workers, and a distinction is now drawn between what are known as the *static* and *dynamic* surface tensions. The static tension is the value obtained, when the determination is carried out on an *old* surface, as with the capillary rise and stalagmometer methods. The term 'surface tension' as generally used in textbooks refers to *static* surface tension. The *dynamic* surface tension on the other hand is the value obtained with determinations on perfectly fresh surfaces, as in the methods of surface tension determination by means of jets, surface ripples, etc. The *dynamic* tension is always illdefinitely that a determination of the static surface tension is insufficient for the purpose of an estimate of the wetting power of a solution containing soap, and this therefore disposes of all the methods as suggested by Brünnich and Smith, Vermorel and Dantony, and Lefroy[1], of comparison of wetting powers by such determinations.

We now come to the question as to how far the weight of liquid, adhering to a strip of a not easily wetted solid, which is immersed in the liquid and then carefully withdrawn, can furnish a criterion of the wetting power of the liquid in question. We have already seen that the power to wet is dependent upon one or both of two main factors: (1) a low surface tension and interfacial tension, and (2) a high surface viscosity. One of the results of a low surface tension and interfacial tension, where surface concentration effects are not very marked, is to cause the liquid to 'run' easily. With tree sprays, this is a most desirable property, in order that the liquid may penetrate the folds and interstices of the leaves etc. In the test of wetting power under consideration, the effect of a low surface tension and interfacial tension would be to wet the surface of the immersed solid completely, from which, on withdrawal from the fluid, all excess of liquid would 'run' off very rapidly, but still a continuous liquid film would remain adhering to the surface. The effect of a high surface viscosity would be in the opposite direction; as soon as ever a thin film of liquid was produced, the surface viscosity would come into play, as a result of which draining would be largely hindered. The amount of liquid adhering to the solid after immersion would therefore be the resultant of two opposite effects, and as such, we should not expect it to differ in any marked degree, whatever the liquid under investigation. Our experiments have proved this actually to be the case. The solid used for immersion experiments was a piece of wide glass tubing, coated internally and externally with a thin layer of collodion. Preliminary trials showed this to be the most suitable surface. Three liquids were used for the purpose of comparison: distilled water, a 1 % saponin solution, and a castor soap solution containing emulsified *green oil*[2]. The liquid under investigation, contained in a large weighing bottle, in order to prevent loss through evaporation, was weighed both before and after the immersion of the solid; the difference in the two weights represented the amount of liquid removed on the surface of the solid. Two experiments with distilled water gave 0·0282 gm. and 0·0356 gm. respectively, as the weight of adhering liquid. Since water does not wet a collodion surface, theoretically no water should be removed by the solid. In actual practice, a variable quantity of water is removed in the form of adherent droplets, and this fact alone would seriously limit the possibilities of the test. With the 1 % saponin solution, which possesses the property of surface concentration to a marked degree, the weight of adhering liquid was 0·0764 gm. With the green oil emulsion, a liquid which had a low surface tension and interfacial tension, and 'ran' well, the corresponding weight was 0·0520 gm. Considering the very great difference in wetting power of the three liquids used, the difference in weight of the adhering liquid is very small, exactly as theoretical considerations had led us to surmise; such differences in weight are altogether too small to serve as criteria of wetting power.

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[1] It may be desirable to point out that Lefroy, after appreciating the fact that the process of wetting is dependent on the relative magnitude of three surface tensions, *viz.*: Wash/Solid, Wash/Air and Air/Solid, has confused their relationship. The condition for wetting is, of course, that the Air/Solid tension should exceed the sum of the other two.

[2] A neutral oil of high boiling point obtained in the distillation of coal-tar.

Lastly, there is the question of *foaming* power. It has been rightly claimed that a solution containing a small quantity of oil in a finely emulsified state, possesses a high wetting power, and it will be seen later why this is so. Does the power of forming a permanent foam afford any indication of the fineness of the emulsion and therefore of the wetting power of the liquid? If, for a moment, we consider those properties upon which foaming and emulsification depend, we shall see that they are but indirectly connected.

Donnan (1899, pp. 43–9) has shown that a soap solution acts as an emulsifier by virtue of its low interfacial tension with respect to the oil. The result of this low interfacial tension is twofold: firstly, the soap becomes concentrated at the surface of the globules of oil, and owing to the surface viscosity produced thereby, the film of liquid intervening between two oil globules resists thinning and the consequent coalescence of the globules. Secondly, the force tending to break the intervening film is that of the interfacial tension, and this being low, is not strong enough to withdraw the aqueous film separating the globules. Hillyer (1903, pp. 516–21), working independently, has arrived at similar conclusions as to the cause of the permanence of emulsions. Stable emulsions of oil in soap solution are therefore dependent upon a low interfacial tension between the oil and the aqueous liquid, and the surface tension of the latter plays no part in the process.

The property of foaming and the factors which lead to the formation of a permanent foam, have formed the subject of a considerable amount of investigation by various workers, amongst whom may be mentioned: Plateau (1873, pp. 1–119), Quincke (1888, pp. 580 et seq.), Rayleigh (1890 *a*, pp. 85 et seq.), Donnan (1899, p. 49), Shorter (1912, pp. 629–32; 1914, pp. 718–20). The generally accepted view of the nature of a foam is that it is an emulsion of air in a liquid. Support is lent to this view by the fact that the requisite conditions for the production of a *permanent* foam are exactly analogous to those necessary for the formation of a permanent emulsion of oil in an aqueous liquid. These we have seen to consist in (1) a high surface viscosity of the intervening film, and (2) an interfacial tension so low as to be incapable of breaking this film. In the case of an oil emulsion, the intervening matrix prevents the coalescence of the oil globules, and similarly, in the case of a foam, it is the intervening matrix which prevents the coalescence of the minute bubbles of air. With emulsions of oil, therefore, it is the interfacial tension of the aqueous liquid to the oil, which is the determining factor; with foams on the other hand, it is the interfacial tension of the aqueous liquid with air, *i.e.* the surface tension of the aqueous liquid, which is of importance. The property of giving a lasting foam therefore indicates that the liquid possesses (1) the property of surface concentration, such as saponin solution, solutions of which show quite exceptional frothing properties; and (2) a low *surface* tension, such as soap solutions. Pure liquids of low surface tension, as benzene, ether, do not give permanent foams. This indicates that surface concentration plays an essential part in the production of foams. It is now evident that, since a fine oil emulsion is dependent upon a low *interfacial* tension between the oil and aqueous liquid, and since foaming is dependent upon a low *surface* tension of the liquid, the power to give a lasting foam gives no indication of the emulsifying properties of the liquid towards oil, and therefore of the state of the oil emulsion contained in the liquid. In other words, foaming power is in no way indicative of a high wetting power. As a matter of fact, one emulsion which we examined, was found to have an extremely low interfacial tension, but the foaming power of the liquid was practically nil.

From the criticism which has been made of the methods hitherto suggested for the comparison of the wetting power of various solutions, it will be evident that the determination of wetting power is fraught with no small difficulty. And so far as liquids, which contain saponin, gelatine and other substances exhibiting surface concentration to a marked degree, are concerned, we do not know of any method by which these difficulties may be overcome. In the case of fluids made up with a soap basis, however, which is the case with most cattle dipping fluids and horticultural sprays, we have worked out a method which affords a convenient and ready means of comparing their wetting powers. The method is a slight modification of that employed by Donnan (1899) for the determination of the emulsifying action of soap solutions. We realise that the method is open to certain objections, and that the results obtained cannot be regarded as absolute. At the same time, we venture to think that it is based on sound theoretical principles, and that the results obtained by its employment are sufficiently accurate for all *practical* purposes.

If we consider once more the equation:

$$T_2 > T_1 + T_{12},$$

it is evident that, if these three tensions could be determined, the expression $T_2 \quad (T_1 + T_{12})$ would represent the wetting power, provided that the disturbing factors of solubility and surface concentration did not come into play. As already stated, the surface tension of a solid and the interfacial tension of a solid to a liquid are indeterminate, from the point of view of a practical test. The interfacial tension between a thick oil and an aqueous liquid can, however, be determined without any great difficulty; and, as the surfaces to be wetted by dips or sprays are usually of a greasy or a waxy nature, there does not appear to be any valid reason why in the comparison of wetting power, a thick oil should not be taken to represent the solid surface.

In our method for the determination of the wetting power of a dip or spray fluid, a thick paraffin oil (liquid vaseline), free from acid, was taken as a standard to represent the greasy surface (*e.g.* a greasy hide, a waxy leaf). The surface tension of this oil was determined once for all by any convenient method, *e.g.* capillary rise. To compare the wetting power of any two preparations (soap), it was then only necessary to determine their surface tension and their respective interfacial tension towards the standard oil, and to substitute these values in the above equation. The values obtained in an actual comparison of the wetting power of two cattle dips are tabulated below. The determinations were made on the liquids diluted with water to the concentration usually employed for dipping, and in this particular instance castor oil was employed as the standard. As the two dips under consideration were both practically neutral in reaction, the use of this oil was permissible, but as a general rule, a neutral mineral oil is preferable.

The values in the last column of Table I indicate that Dip A should have the power of wetting a surface made greasy with castor oil, whilst Dip B should lack this power. This was found actually to be the case. A glass slide, smeared with a thin layer of castor oil, was completely covered and wetted when the dilution of Dip A was poured over it, whilst a similarly prepared slide, on being treated in the same manner with the dilution of Dip B, was found to assert its greasy nature, the dipping fluid running into drops and failing to wet[1].

TABLE I. *Wetting Power of Cattle Dipping Fluids towards Castor Oil.*

Dip	Surface tension of diluted dip T_1	Surface tension of oil T_2	Interfacial tension of oil and diluted dip T_{12}	$T_2 - (T_1 + T_{12})$ – wetting power
A.	34·6 dynes per cm.	38·1 dynes per cm.	0·4 dynes per cm.	+ 3·1 dynes per cm.
B.	40·8 dynes per cm.	38·1 dynes per cm	10·0 dynes per cm.	– 12·7 dynes per cm.

By methods to be described later, we have determined the surface tension, and the interfacial tension towards liquid vaseline, of solutions at various concentrations of most of the commercial soaps usually employed in the preparation of dips and sprays. There is no particular point in the publication of all these results. These various determinations were carried out to ensure that this method of comparing wetting power is applicable to all kinds of soap. As the results obtained with the different soaps did not vary markedly from one another, those given by sodium oleate are tabulated below and may be taken as typical. As commercial soaps vary so greatly in composition, the concentration of soap is expressed as percentage of fatty acid. The determinations were all carried out at a uniform temperature of 20° C.

Owing to the low surface tension of the liquid vaseline employed, the values obtained for the expression $T_2 - (T_1 + T_{12})$, *i.e.* the wetting power, are all negative. This indicates that the soap solutions used are incapable of wetting the vaseline, which actual trial with a slide smeared with vaseline showed to be the case. The fact that the vaseline is not wetted by soap solution, however, in no way precludes its use as a standard oil in the determination of wetting power.

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[1] Of course, in time both dips would wet the *glass* owing to the displacement of the oil from the glass surface. What we are at present interested in is the power of the dips to wet the *surface* of the oil.

TABLE II. *Wetting Power of Sodium oleate Solution towards liquid Vaseline (at 20° C.).*

Concentration	No. of drops	Surface tension of soap solution T_1	Surface tension of oil T_2	Interfacial tension of oil and soap solution T_{12}	$T_2 - (T_1 + T_{12})$ = wetting power
		dynes/cm.	dynes/cm.	dynes/cm.	dynes/cm.
2·0 % F.A.	--*	31·00	31·11	1–2	−1–2
1·0	1300	29·35	31·11	5·87	−4·11
0·5	1134	28·61	31·11	6·73	−4·23
0·25	730	28·28	31·11	10·46	−7·63
0·10	453	28·78	31·11	16·85	−14·52
0·05	274	31·34	31·11	27·86	−28·09
0·01	141	33·75	31·11	54·14	−56·78
0·001	119	55·00	31·11	61·14	−85·03
0·0001	117	70·99	31·11	65·24	−105·12

* This concentration 'streamed' in our usual drop-pipette. The interfacial tension was determined therefore in a pipette with a finer orifice. The number of drops is not given, as it is not comparable with the other figures.

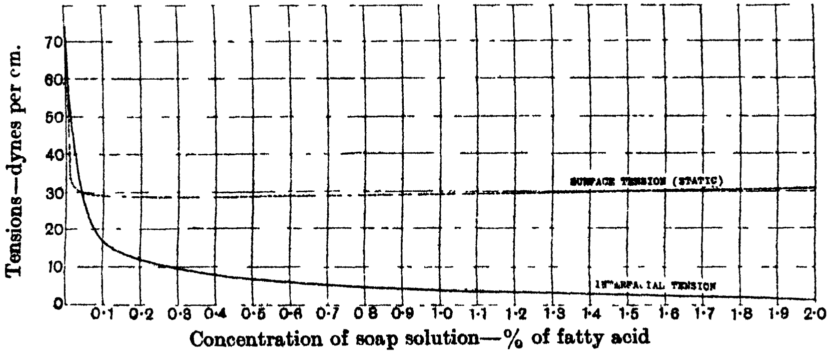

Fig. 2.

The values for the surface and interfacial tensions are plotted in Fig. 2; the tensions as abscissae, the concentrations of fatty acid as ordinates. The forms of the two curves are markedly different. With concentrations of more than about 0·1 % of fatty acid, the surface tension is only very slightly affected by rising concentration, the tendency being to increase rather than decrease; the interfacial tension, on the other hand, decreases progressively. This difference is evidently to be explained by surface concentration phenomena, which are not nearly so marked at the interface of the oil and soap solution as at that of the soap solution and air. We have carried out exactly similar determinations on solutions of castor oil, sulphonated castor oil, linseed oil, resin and commercial soft soaps, and in every case we have found that, whilst the surface tension, at concentrations higher than 0·1 % of fatty acid, has shown but little variation with the concentration, the interfacial tension has decreased progressively up to about 2 % of fatty acid, when the interfacial tension usually approaches zero.

It is for this reason that in the actual practical method of comparison of the wetting power of dips and sprays, we do not recommend the determination of the three tensions T_1, T_2 and T_{12}.

It is obvious from the results given in Table II and illustrated graphically in Fig. 2, that of these three tensions, it is the interfacial tension (T_{12}) which exerts the predominating influence, so far as wetting is concerned. It is for this reason, coupled with the fact that the interfacial tension is the least affected of any by surface concentration effects, that we consider that the interfacial tension forms a better criterion of the wetting power than the expression $T_2 - (T_1 + T_{12})$, which, since it contains T_1, is itself vitiated by such concentration effects.

The method which we have employed for the determination of surface tension of the soap solutions (see Table II) is Serle's Torsion Balance Method (1913) which proved very convenient for the purpose. It is rapid and fairly accurate, and, where a large number of comparative determinations have to be made, can be confidently recommended. As, however, a determination of the surface tension is not necessary in the method of comparison of wetting power which we advocate, it is beyond the scope of this paper, and the reader is referred to the original communication.

Our method of estimating wetting power merely resolves itself into a determination of the interfacial tension of a standard thick paraffin oil with the liquid under investigation. There are several methods by which this interfacial tension may be measured, but the one most usually adopted now is Donnan's (1899) drop-pipette method; and, after some preliminary trials with most of the other methods, it quickly became evident that Donnan's method was the only one suitable for our purpose.

In this method, the oil is run from a pipette through the aqueous liquid, and the number of drops formed from a definite volume of oil are counted. The size of the drop and hence the drop number is

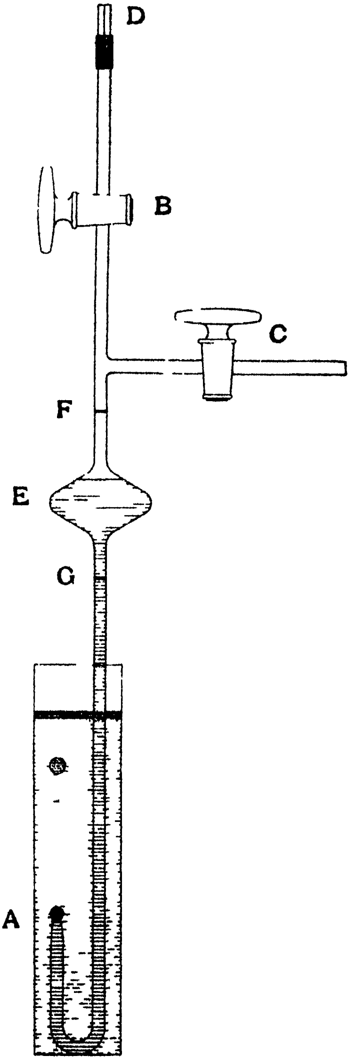

Fig. 3.

dependent upon (1) the interfacial tension, (2) the size of the aperture of the pipette, (3) the difference in density between the oil and the aqueous liquid, and (4) a constant depending upon the size of the aperture, the difference in density and the interfacial tension. Where it is desired to state the interfacial tension in absolute terms, all these four factors must be taken into account. The calculations involved are somewhat tedious; they have been dealt with fully by Lewis (1908), to whose paper those interested are referred. The values given in Table II for the interfacial tension were obtained by this method.

Where comparative values only are required, as is the case in the determination of wetting power, the interfacial tension may be taken as inversely proportional to the number of drops, since the diameter of the aperture remains constant, and the difference in density between the oil and the aqueous liquids does not usually vary greatly, owing to the high dilution at which the dip or spray fluid is employed. As we have already seen that the wetting power varies indirectly as the interfacial tension, it follows that the wetting power is directly proportional to the drop number.

The form of pipette used for the determination of the interfacial tension is shown in Fig. 3, which requires little explanation. The lower limb (*A*) is curved round, and its aperture is somewhat restricted and ground carefully to a plane surface. The walls of the aperture should be fairly thin, in order to prevent unevenness in wetting. Both the upper limb of the pipette, as well as the lateral tube with which it is provided, are closed with glass stop cocks (*B* and *C*). The lateral tube is used for filling the pipette by suction. The upper limb terminates in a very fine capillary tube (*D*), which is best attached by means of rubber tubing. The object of this capillary is to regulate the flow of oil through the aqueous liquid, and should be so selected as to allow an interval of about ten seconds between successive drops, when water is used.

The volume of the pipette used in our experiments was 49·1 c.c., the length from the bottom of the bulb (*E*) to that of the bend 22·5 c.c.; the diameter of the aperture was 3·5 mm. Later experience has indicated that a pipette with a capacity of 25 c.c. and an aperture 2·7 mm. in diameter would probably be found most convenient for general purposes. The paraffin oil used as a standard was a liquid vaseline, which was almost acid-free and possessed a density of 0·8690[1].

An actual determination is carried out as follows. The preparation (*e.g.* dipping fluid) is diluted[2] to the strength at which it is generally used in practice, and 250 c.c. of the diluted liquid are placed in a gas jar (20 cm. high × 5·5 cm. internal diameter). The pipette is then filled to above the upper mark (F) with oil, and, after carefully wiping the outside of the pipette and the surface of the orifice, the pipette is lowered into the gas jar and allowed to rest on its bottom. The pipette is maintained in a vertical position by means of a clamp. The top stopcock is then opened and the level of the oil allowed to sink to the *upper* mark. Any oil adhering to the orifice is carefully removed by means of a glass rod, after which the stopcock is again opened and the number of drops formed whilst the level of the oil in the pipette sinks to the *lower* mark (G) are counted.

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[1] Obtained from the Chesebrough Manufacturing Co., Holborn Viaduct.

[2] In order to obtain comparable results, the dipping fluid should always be diluted immediately before the count is made.

It may happen, with liquids of very low interfacial tension, that the oil flows from the orifice in a continuous stream instead of forming drops. This occurs when the interfacial tension is so low that the diameter of the drops is less than that of the orifice of the pipette, and with our pipette, it signified an interfacial tension of less than 0·66 dynes/cm. If streaming should occur, a pipette with a smaller aperture may be employed or the aqueous liquid must be further diluted.

It is advisable to keep a good stock of standard oil so that all determinations may be made on the same sample of oil and therefore be strictly comparable. The same lot of oil should not be used for more than one determination. As different samples of liquid vaseline differ slightly and give somewhat different drop numbers with distilled water, it is advisable to take the latter as a standard liquid, and to express the wetting power of the solution as the ratio of the drop number of the solution to that of distilled water, multiplied by 100. The following table contains the results of determination of the wetting power of different cattle dips. The dips were all diluted to the same degree, and the drop number determined at a temperature of 20°.

TABLE III. *Comparison of the Wetting Power of Cattle Dips.*

Solution	Drop number	Wetting power = $\frac{\text{Drop no. solution} \times 100}{\text{Drop no. water}}$
Dip A	585	380
„ B	290	184
„ C	543	353
„ D	386	251
Distilled water	154	—

We do not claim that this means of estimating wetting power is free from every objection, nor can it be regarded as affording absolutely accurate values. The interfacial tension existing between an oil and an aqueous liquid is itself a somewhat indefinite quantity, and varies with the age of the oil globule. At the same time, we feel that the method will furnish a means, sufficiently accurate for the purpose, of determining and expressing *in numerical values* the wetting power of a solution, and therefore will supply a long felt need. The apparatus required for the test is simple, and at the same time there is no call for great manipulative skill in carrying out the determination. If attention is given to the few simple precautions mentioned, comparable and reliable results are easily obtained. It may be desirable to mention once again that the method is only applicable to preparations containing *soap* as a basis; with other bases, *e.g.* gelatine, saponin, etc., it entirely fails. With these substances, surface concentration at the interface of the oil and aqueous solution becomes so marked that quasi-solid surfaces are produced, which completely vitiate the drop number and therefore the value for the interfacial tension. We are at present endeavouring to find a simple method by which the wetting power of solutions of such substances may be compared, but up to the present have been unable to evolve anything satisfactory.

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(*Received June 7th*, 1915.)

SOIL GASES.

By J. WALTER LEATHER, V.D., F.I.C.

(Imperial Agricultural Chemist, Agricultural Research Institute, Pusa, Bengal).

At page 5 of their recent communication on the composition of soil gases[1] Messrs Russell and Appleyard give some data regarding gas which was extracted from portions of soil which had been placed in flasks, the air pumped out and the soil then allowed to stand for one or more days. The authors state that "the total amount of gas given up is not great." As a matter of fact a little consideration shows that the volume of this gas was indeed comparatively very large.

This gas may be compared with that originally included in the soil mass. Judging from the data given on page 44, the "free air" varied from 8 to 18 c.c. per 61·8 c.c. or (61·8 × 2·31) = 143 grams soil, or from 5·6 c.c. to 12·6 c.c. per 100 grams dry soil. Reference to Table III shows that the gas which was obtained from the several portions of soil, subsequent to the removal of the air of the flasks, was considerably greater than the "free air" of the soil.

Considering secondly the composition of this gas, it is evident that its origin is certainly not accidental. The authors state that it consists "mainly of carbon dioxide with some nitrogen." Here again the figures hardly support such a definition, for the nitrogen exceeds the carbon dioxide in a number of the samples. Again, although to a certain extent the carbon dioxide could be assumed as having been derived from calcium bicarbonate solution in the soil, its quantity is very far greater than could be present in this form. The water present could only hold a certain amount of calcium bicarbonate in solution, and it is from this item that the above deduction can be made. Lastly the volume of nitrogen obtained is very far in excess of what could be assumed to be condensed or dissolved on soil particles.

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[1] This *Journal,* Vol. VII. pp. 1–48.

I have subjected the Pusa soil to the same treatment as that employed by Russell and Appleyard. A portion of soil, which was subsequently found to include 382·8 grams dry soil and 32·8 grams water, was placed in a bottle and the air pumped out. It was then left for four days connected with the Töpler pump. At the end of the first 24 hours a few c.c. of gas were obtained, after a further 24 hours another small quantity was removed, and on the fourth morning a much less quantity was present. The total gas obtained measured 7·27 c.c. and included carbon dioxide 5·62 c.c., oxygen 0·10 c.c. The volume of gas originally present in this soil would be about 100 c.c.

In other two experiments the soil was taken in the special tool which I have devised for the examination of soil gases. In this case the gases are removed from the *undisturbed* soil. The volume of soil was 154·4 c.c. in each case. In the first experiment 61·19 c.c. of gas was removed at once. After standing for 48 hours 1·87 c.c. of gas was removed, of which 0·87 c.c. was carbon dioxide. In the second of these experiments the gas first removed was 52·33 c.c. After standing for 48 hours, 2·80 c.c. more gas was obtained, of which 2·18 c.c. was carbon dioxide. It is evident that this soil behaves differently from the Rothamsted soil. The undisturbed soil yields after 48 hours a small quantity of gas, most of which was probably present when the sample was taken; but the soil after disturbance yields a small amount of a gas mixture which is qualitatively similar to that obtained at Rothamsted, but is comparatively very small in quantity.

Soils will no doubt vary in respect of the amount of gases which their solid constituents can condense, but I may here mention that the soil of this part of India condenses so little that it is difficult to estimate it; it is certainly less than 1 % of the whole of the gases present in the soil. Details regarding this and other features of the soil gases present in the land of this Institute are about to appear as a memoir.

One is forced to the conclusion that the gas which was obtained on successive days by Russell and Appleyard was *formed* and liberated gradually, presumable by bacterial action.

PUSA,
9th June, 1915.

REPLY TO DR LEATHER.

In our paper we did not discuss Table III in any detail because as we stated the dissolved atmosphere in the soil is under further investigation. It is sufficient to say that the gas was not obtained in the manner suggested in the opening lines of the above note. The dissolved gas begins to come out directly the pressure is lowered by the working of the pump, and no sharp line can be drawn to indicate precisely what fraction of the extracted gas was free and what dissolved. For this reason we refrained from making any estimate of the amount of the dissolved atmosphere except to say that it is small. No comparison can be instituted between the data in Table III and those on page 44 owing to the difference in experimental conditions, and, moreover, the first and second lots of 30 c.c. recorded in Table III, on the composition of which Dr Leather bases part of his argument, certainly contained some of the free air. The successive extractions were not made on successive days: in some cases there had been practically no interval between them.

The conclusion we drew from the experiments was that the atmosphere dissolved in the surface films of water and other substances is almost devoid of oxygen and consists mainly of carbon dioxide with some nitrogen. This seems to us to be a legitimate deduction from the figures, and we do not think we can go any further at present. Dr Leather says that the Pusa soil behaves differently. The figures he quotes, however, seem to lead to the same conclusion: in his case also the amount of gas is small and it appears to be poor in oxygen. Where then is the difference?

A. APPLEYARD.
E. J. RUSSELL.

ROTHAMSTED EXPERIMENTAL STATION,
HARPENDEN.

(*Received June 29th*, 1915.)

ON OVARIOTOMY IN SOWS; WITH OBSERVATIONS ON THE MAMMARY GLANDS AND THE INTERNAL GENITAL ORGANS.

BY K. J. J. MACKENZIE, M.A., AND F. H. A. MARSHALL, Sc.D.
(School of Agriculture, Cambridge.)

PART IV.

IN Part III[1] of this series of communications we recorded that no mammary pigment could be found in the old sows that were examined by us, and we suggested the possibility that such pigment had previously been present and had been destroyed or removed during the periods of glandular activity.

In the present note we record the results of four experiments which were undertaken to test this hypothesis.

Four sows of coloured varieties were taken, three being Large Blacks and one a Berkshire. Each in turn was placed upon an operating table and anaesthetised. Incisions were then made with a scalpel in the tissue of two of the mammary glands, each cut being sufficiently deep and extensive to reveal the existence of mammary pigment if such were present in the neighbourhood of the nipple. After the examination had been completed, the incised tissue was stitched up and dressed with cotton wool and collodion. The wounds healed by first intention. The four sows were all operated upon on the same day, October 15th, 1913.

The following are the details of the experiments together with the further history of each of the sows.

(1) In a Large Black sow two mammary glands were examined for pigment, namely those associated with the first (i.e., the most anterior) nipple on the right side, and the third nipple on the left side. Black pigment could readily be detected in each of the two glands. The sow

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[1] This *Journal*, Vol. VI. May, 1914, p. 182.

subsequently had two litters, each of eight pigs, which were suckled. The first litter was born on March 29th, 1914, and the second on October 13th, 1914. On February 15th, 1915, the sow was killed, when all the mammary glands (including those previously examined) were closely searched for pigment. None whatever could be found.

(2) In a Large Black sow two glands were examined for pigment in the same manner as that adopted in the first experiment. The first gland on the right side did not appear to contain pigment, but in the fifth gland on the other side some was found a little distance from the nipple. The sow had a litter of nine on March 22nd and a litter of eleven on October 8th, 1914, and lactated normally. When the animal was killed on February 15th, 1915, not a trace of pigment was found in any of the glands which were carefully examined.

(3) In a Berkshire sow the right anterior gland was found to contain a large quantity of pigment, but none was found in the fourth gland on the left side. The sow on April 14th had a litter of six pigs and on October 13th a litter of five pigs which were suckled normally. On killing the sow on February 15th, 1915, no mammary pigment could be detected.

(4) In a Large Black sow the first right and third left glands were examined and each was seen to possess much pigment. The sow produced a litter of nine young on March 23rd, and a litter of eight on October 15th. Both were suckled. On killing the animal on February 15th, 1915, a very slight trace of dark pigment was found just below the first teat on the left side, but none in association with either of the other teats, so it was evident that pigment had been removed from the glands previously searched.

Each of the sows at the time of killing had large protruding follicles in the ovaries, indicating the approach of another heat period, and there was every indication that the animals were sexually normal.

The above described experiments prove very clearly that the dark pigment which so frequently occurs in the mammary tissue in pigs of coloured breeds, and which we have shown to exist even in the embryo, may be no longer present in sows which have been bred from. The precise period (or periods) at which this pigment disappears is still unknown, but there is a strong presumption that its removal takes place either during the progress of lactation, or in the period of pregnancy when the mammary glands are being built up preparatory to the secretion of milk. The fact that mammary pigment can become diminished in quantity to the point of disappearing altogether, is a point of great importance to the investigator who might otherwise be in danger of concluding that certain individual sows belonged to a strain which did not carry mammary pigment, when in reality he would be dealing with cases in which such pigment had formerly been present in quantity and had subsequently been removed.

The operations were performed at the Field Laboratories, Milton Road, Cambridge, by F. H. A. Marshall.

The expenses of the investigation were defrayed by the Board of Agriculture and Fisheries out of funds allocated to them for purposes of research, by the Development Commission

(*Received July 23rd,* 1915)

THE EFFECT OF REMOVING THE SOLUBLE HUMUS FROM A SOIL ON ITS PRODUCTIVENESS.

By WILLIAM WEIR, M.A., B.Sc.
Carnegie Research Scholar.

(*Rothamsted Experimental Station.*)

The decay of plant and animal residues in soils produces brown organic compounds commonly designated as humus. Some of it is soluble in dilute alkaline solutions, and agricultural chemists and investigators have often assumed that this part plays an important function in the nutrition of plants by reason of its solubility. As a result numerous methods have been devised for determining this readily soluble material, and its amount has been regarded as a measure of the fertility of the soil. Since however the amount of nitrogen in the soluble humus material is commonly 40 per cent. to 50 per cent. of the total nitrogen, the results for ordinary soils gave no better indication of potential fertility than did the total nitrogen determination itself[1].

Few experiments seem to have been made to ascertain directly if the soluble humus really does play any considerable part in plant nutrition. The only one that has been found is recorded by Grandeau[2] in 1872. A kilogramme of Russian Black Earth was divided into two parts; one was treated with dilute hydrochloric acid and ammonia solution to remove soluble humus, or, as he termed it, *matière noire*; the other was left untreated.

The two lots of soil were put into flower pots and watered to saturation point with distilled water, the untreated soil taking 7 per cent.

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[1] "Results of Investigations on the Rothamsted Soils," 1902, by Bernard Dyer. N. H. J. Miller's "Determinations," p. 179.

[2] "Recherches sur le rôle des matières organiques du sol dans les phénomènes de la nutrition des végétaux," par M. L. Grandeau. *Publication de la Station agronomique de l'Est.* 1872.

more water than the other. Beans were planted in both: they developed normally on the untreated soil but failed entirely on the extracted soil.

During the past two seasons this experiment has been repeated on a larger scale, with results sufficiently interesting to justify their being placed on record. The removal of the soluble humus was effected by washing the soil with dilute hydrochloric acid to remove bases, and then repeatedly extracting with dilute soda. Some 40 per cent. of the nitrogen in the soils was thereby removed.

Vegetation experiments were then conducted with large quantities of untreated and extracted soils, and approximately equal total yields both of dry matter and of nitrogen were obtained over four successive crops. Thus it appears that the removal of the soluble humus had no effect in diminishing the productiveness of the soil in spite of the fact that the soil used was known to respond to nitrogenous fertilisers.

Laboratory experiments were then started to ascertain the effect of removing soluble humus on the rates of production of nitrate and ammonia in the soils. Here the indications were that the removal of the soluble humus increased the amount of ammonia but diminished that of nitrates in the soil, and the sum of ammonia and nitrate was usually less than in the untreated soil. The numbers of bacteria however were considerably increased. No marked difference was produced where 0·5 per cent. untreated soil was added to replace the bacterial flora that might have been destroyed by the acid and alkali treatment. These results are not necessarily inconsistent with the vegetation experiments. It has been shown in these laboratories that the accumulation of ammonia and nitrate in an uncropped soil will only proceed to a certain stage when it stops[1]. In vegetation experiments on the other hand the nitrate is perpetually being removed by the growing plant. There is thus a considerable difference in conditions.

Method of Extraction. 250 grams of air-dried soil, which has passed through a 3 mm. sieve, are shaken up with 2500 c.c. N/5 HCl. After standing in contact with the acid for one hour, the soil is thrown on to a filtering funnel and washed free of acid; 500 grams of soil treated in this manner are shaken with 2000 c.c. 2 per cent. NaOH and allowed to settle overnight. The dark-coloured humus extract is siphoned off. This process is repeated five times in all, and at the fifth extraction the liquid is of a lightish brown tint. The soil is then shaken with water, allowed to settle, the supernatant liquid is removed, and finally the whole is made slightly acid with HCl to promote flocculation and allow of easy filtration and washing. The acid is then removed, the soil dried and passed through a 3 mm. sieve.

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[1] "The Effect of Partial Sterilisation of Soil on the Production of Plant Food," by E. J. Russell, and H. B. Hutchinson. *Journal of Agricultural Science*, v. p. 193.

The soils used were: (1) Allotment soil, a medium garden soil; (2) Harpenden Field Soil (Arable), a typical loam. The treatment was found to reduce the nitrogen very considerably.

TABLE I.

Total Nitrogen in Untreated Allotment Soil, per cent.	·315
" " in Extracted " " "	·178
" " removed, per cent.	·137
Percentage reduction	43·6
Total Nitrogen in Untreated Field Soil, per cent. ...	·186
" " in Extracted " " " ...	·109
" " removed, per cent.	·077
Percentage reduction	41·2

Vegetation experiments were made with the four soils (Untreated Allotment, Extracted Allotment, Untreated Field Soil, and Extracted Field Soil respectively).

Each pot had a capacity of 10 kilos.; 5 kilos. sharp sand were put at the bottom, and above this 5 kilos. of the soil under investigation, mixed with 15 per cent. of sharp sand. Each set consisted of 5 pots: thus there were 20 pots in all. Chalk was added to replace carbonates removed by the acid treatment and 1 gram KH_2PO_4 in solution was added to each pot. Thus the soils were as follows:

TABLE II.

	Untreated Allotment	Extracted Allotment	Untreated Harpenden	Extracted Harpenden
Fine Earth ...	5000 grams	5000 grams	5000 grams	5000 grams
Sand	750 "	750 "	750 "	750 "
Chalk	—	21 "	—	47·5 "
KH_2PO_4	1 gram	1 gram	1 gram	1 gram

Wheat was sown on March 14th, 1914, and the pots were kept in the glass-house till April 7th when the number of plants was reduced to five per pot and all the pots were removed to the open. The plants in all cases made slow growth; those in the untreated soils tillered better, but later in the growing period were caught up, and passed by those in the treated soils. Subsequent growth was quite satisfactory, and in August, when the crops were cut, the weights were:

TABLE III. *Weights of first crop, Wheat.*

Soil	Weight of Wheat dried at 100° C.	Percentage of Nitrogen in crop	Weight of Nitrogen in crop
	grams		grams
Allotment Soil	67·8	·95	·64
Allotment Soil Extracted	72·3	1·01	·73
Harpenden Field Soil	45·3	·82	·37
Harpenden Field Soil Extracted ...	67·2	1·06	·71

After the wheat was cut the soils were thrown out of the pots, mixed up afresh and replaced as before. Mustard was then sown (still in August), during rather dry weather, and was grown in the open. Only on Harpenden Field Soil was any appreciable yield obtained: on the extracted soil the plants scarcely grew beyond the seed-leaf stage. On both allotment soils the crops were poor. The mustard was pulled on October 8th and dried and weighed as before.

TABLE IV. *Weights of second crop, Mustard.*

Soil	Weight of Mustard dried at 100° C.	Percentage of Nitrogen in crop	Weight of Nitrogen in crop
	grams		grams
Allotment Soil	2·7	2·34	·06
Allotment Soil Extracted	3·9	2·51	·08
Harpenden Field Soil	13·1	1·93	·25
Harpenden Field Soil Extracted ...	·9	3·44	·03

TABLE V. *Weights of third crop, Rye.*

Soil	Weight of Rye dried at 100° C.	Percentage of Nitrogen in crop	Weight of Nitrogen in crop
	grams		grams
Allotment Soil	3·1	3·94	·12
Allotment Soil Extracted	3·7	3·68	·14
Harpenden Field Soil	5·4	3·66	·20
Harpenden Field Soil Extracted ...	6·6	4·17	·28

The soils were then cultivated but not otherwise disturbed. On October 13th all the pots were sown with rye, which grew in the glass-house till 18th February, 1915, when the plants, 10 to each pot, were pulled, as it seemed unnecessary to allow them to ripen. The crops were dried, weighed, and the nitrogen determined as before. The soil in all the pots was thrown out and thoroughly dried before replacing in the pots and sowing with a fourth crop—mustard.

Mustard was sown in all the pots in April, 1915, and was left till the end of May when it was pulled, dried and weighed. There was a fair crop on all the soils except on the Extracted Harpenden Field Soil, where it failed entirely. The plants were cut when just flowering.

TABLE VI. *Weights of fourth crop, Mustard.*

Soil	Weight of Mustard dried at 100° C.	Percentage of Nitrogen in crop	Weight of Nitrogen in crop
	grams		grams
Allotment Soil	11 3	2·12	·24
Allotment Soil Extracted	10·8	2·19	·24
Harpenden Field Soil	15 4	1·97	·30
Harpenden Field Soil Extracted ...	1 3	3·53	·05

Table VII gives a summary of the crop and nitrogen data for the different soils.

TABLE VII. *Summary of weights of all four crops.*

Soil	Weight of Wheat	Nitrogen in Wheat	Weight of Mustard	Nitrogen in Mustard	Weight of Rye	Nitrogen in Rye	Weight of Mustard	Nitrogen in Mustard	Total Weight of Dry Matter produced	Total Weight of Nitrogen removed in four crops
	gms.	%	gms.	%	gms.	%	gms.	%	gms.	%
Allotment Soil	67 8	·95	2·7	2 34	3 1	3·94	11·3	2·12	84 9	1·07
Allotment Soil Extracted	72·3	1·01	3·9	2·51	3·7	3·68	10·8	2·19	90·7	1·19
Harpenden Field Soil	45 3	·82	13 1	1·93	5·4	3·66	15·4	1·97	79·2	1·13
Harpenden Field Soil Extracted	67·2	1 06	·9	3·44	6·6	4·17	1·3	3·53	76·0	1·07

Thus it will be seen that in five cases out of eight the plants obtained more nitrogen from the extracted than from the untreated soils, in one case the amount was the same and in two cases the amount was less but on both these occasions the crop (mustard) failed on one soil.

TABLE VIII. *Nitrogen in soils and crops.*

Soil	Percentage of Nitrogen in Soil	Total Weight of Nitrogen per pot	Total Nitrogen removed in four crops
		grams	grams
Allotment Soil	·315	15·74	1·07
Allotment Soil Extracted	·178	8·88	1·19
Harpenden Field Soil	·186	9·28	1·13
Harpenden Field Soil Extracted ...	·109	5·46	1·07

During the winter of 1914–1915 additional quantities of the same two soils were extracted and used in vegetation experiments in precisely the same way as in the previous sets. In this instance the acid and alkali treatment removed a somewhat smaller amount of the soluble humus matter from both soils.

TABLE IX.

Total Nitrogen in Untreated Allotment Soil, per cent.	·326
„ „ in Extracted „ „ „ ...	·206
„ „ removed, per cent.	·120
Percentage reduction	36·8
Total Nitrogen in Untreated Field Soil, per cent. ...	·177
„ „ in Extracted „ „ „ ...	·107
„ „ removed, per cent.	·070
Percentage reduction	39·5

In the case of the Allotment Soil, wheat was sown in the pots on November 12th, 1914. The plants in the untreated soil grew and tillered better than the others but in both sets the crops were good. They were cut when nearly in full ear on 23rd June, 1915.

Wheat was not sown in the Harpenden soils till 25th March, 1915, so that the growing period was much the same as for the wheat crops of the previous season. It was cut on 23rd June, 1915, when the ears were beginning to come out.

The yields were comparatively poor but during the whole time the plants in the extracted soil were greener in the leaves and did better than those in the untreated. The following figures were obtained for the four soils on cutting and drying:

TABLE X.

Soil	Weight of Wheat dried at 100° C.	Percentage of Nitrogen in crop	Weight of Nitrogen in crop
	grams		grams
Allotment Soil	148 9	·82	1 22
Allotment Soil Extracted	127 7	·82	1·03
Harpenden Field Soil	47 8	1·18	·56
Harpenden Field Soil Extracted ...	57·7	1·25	·72

Experiments on the rate of nitrate production in the soil. These were made with Untreated and Extracted Allotment Soils respectively, and they involved periodical determinations of nitrate, ammonia, and numbers of bacteria. Chalk was added to replace the carbonates removed during extraction, and a third set was put up consisting of extracted soil plus carbonate, seeded with 0·5 per cent. of untreated soil to replace the bacterial flora that might have been destroyed by the acid and alkali treatment. Estimations were made at the start, after one week, after three weeks, and after a considerable period. The soils were stored in bottles, and the moisture content adjusted to approximately 16 per cent., this being known as favourable for bacterial action. Table XI shows the changes that occur.

It will be seen that the formation of nitrate is less marked in the treated soils, and that ammonia tends to occur in quantity and to persist, whilst the untreated soil contains only the normal three or four parts per million. Further, while the numbers of bacteria in the untreated soil are normal throughout--about 20 millions per gram downwards—the numbers in the extracted soils are initially very low, then become abnormally high and remain above the usual level; there is however no corresponding increase in the production of ammonia and nitrate. This is very similar to the result obtained by W. Buddin[1] on treating soils with non-volatile antiseptics. Sowing the extracted soil with bacteria from untreated soil causes no marked difference in the results.

[1] *Journal of Agricultural Science*, 1914, vi. pp. 417-451.

TABLE XI. *Changes in bacterial numbers, in ammonia and nitrate, in soils before and after extraction of soluble humus.*

Time	Untreated Allotment				Extracted Allotment + $CaCO_3$				Extracted Allotment + $CaCO_3$ + ·5 % Seeding			
	Parts per million			Millions per gram	Parts per million			Millions per gram	Parts per million			Millions per gram
	Nitrate	Ammonia	Water %	Bacteria	Nitrate	Ammonia	Water %	Bacteria	Nitrate	Ammonia	Water %	Bacteria
May 4th, 1914.												
Initial	6	2	16	12	8	7	2	—	8	8	2	—
After 1 week ...	15	—	16	2	9	7	14	34	10	9	14	31
After 3 weeks...	10	3	16	3	9	8	14	17	12	8	14	16
After 37 weeks	50	2	14	7	19	7	13	23	26	2	13	46
After 43 weeks	58	—	14	5	32	2	13	39	29	2	13	34
November 26th, 1914.												
Initial	16	4	18	18	7	15	2	1				
After 1 week ...	25	3	18	28	18	18	16	119				
After 3 weeks...	39	3	18	10	21	15	16	167				
After 16 weeks	38	1	17	11	22	19	15	62				
February 3rd, 1915.												
Initial	16	3	21	5	7	16	2	—	8	18	2	—
After 1 week ...	17	4	18	15	9	15	16	81	11	19	17	106
After 3 weeks...	24	1	21	17	13	15	17	63	12	17	18	107
After 14 weeks	39	2	21	11	13	17	17	48	30	14	16	40

(*Received July 26th,* 1915.)

VOLUME VII FEBRUARY, 1916 PART III

STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

I. THE CARBOHYDRATES OF THE MANGOLD LEAF.

BY WILLIAM A. DAVIS, ARTHUR JOHN DAISH
AND GEORGE CONWORTH SAWYER.

(Rothamsted Experimental Station.)

INTRODUCTION.

THE object of the investigations recorded in the present series of papers was to throw light on fundamental problems—how carbohydrates are formed in the foliage leaves of plants, how they are transferred to the reservoirs where they are stored (as saccharose in the sugar beet or mangold[1], as starch in the potato and the cereal crops and as inulin in many Compositæ such as the artichoke or dahlia) and how they are finally broken down and utilised in subsequent growth. A complete account of the work done in this field up to the year 1893 was given by Brown and Morris [1893] in their classical paper on "The Chemistry and Physiology of Foliage Leaves."

Sachs [1862] first proved that the production of starch in the chlorophyll granule depends on the action of light and that the starch formed during the hours of sunlight is wholly or partially redissolved and removed from the leaf during the night to supply the demands of the growing points of the plant. Sachs regarded the starch as the "first visible product of assimilation" and considered that *all* the carbohydrate synthesised in the leaf passed through the starch stage; he was of opinion that the starch disappeared in the form of sugar. Schimper [1885] on the other hand held that starch is not only converted into sugar in the plant but, from his observations of the increase of starch in leaves supplied artificially with solutions of sugar, concluded that glucose precedes starch in the process of assimilation, the starch being formed from the glucose when the concentration of the latter exceeds a certain maximum which differs in different plants. Taking into account Baeyer's hypothesis, advanced in 1870, it was possible to regard the glucose as formed by the polymerisation of formaldehyde, CH_2O, which was itself produced directly by the reduction of carbonic acid in the leaf under the influence of light and chlorophyll.

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[1] Both the sugar beet and the mangold are varieties of *Beta vulgaris* L. and apparently have been derived by cultivation from the *Beta maritima* of our coasts.

Some such view as this appears to have been generally held down to 1893, although Arthur Meyer [1885] emphasised the fact that the formation of starch in plants was by no means universal[1]; many monocotyledons, particularly the *Liliaceæ*, form very little starch in their leaves, some none at all (e.g. *Allium cepa*, *Scilla maritima*). An important point established by Meyer was that leaves of plants which store starch abundantly contain comparatively little sugar, whilst plants like *Gentiana lutea*, *Allium* and Asphodel, which form little or no starch in the leaf, accumulate large stores of soluble reducing substances, probably glucose. When starch is absent Meyer considers that its place may be taken as a reserve substance by other sparingly soluble carbohydrates, such as inulin (*sinistrin*), which he isolated from the leaves of the Yucca. In 1886 Meyer [1886] made the interesting observation that almost all leaves which are capable of forming starch at all, produce it abundantly from a 10 per cent. solution of laevulose and a relatively small number from dextrose.

The important work of Brown and Morris in 1893 may be regarded as marking the beginning of a new period in the study of the physiology of the leaf. Up to this date, the chemical methods used had been largely qualitative or very roughly quantitative; little attempt had been made to discriminate between the reducing sugars of the leaf, which were universally regarded as glucose (dextrose). The possibility of laevulose (fructose) being present or a mixture of laevulose and dextrose (invert sugar) had hardly been raised, whilst maltose had not been suggested as a possible leaf carbohydrate, although it was known that this sugar was formed from starch by the action of diastatic enzymes. Cane sugar, although known to be widely present, had not been taken into account as a possible direct product of photosynthesis (except by A. Girard [1884] in an important paper referred to later); it was generally regarded as a reserve carbohydrate formed as a secondary product from the simpler sugars.

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[1] Boehm [1856] had found that the chloroplasts of *Allium* species, *Galanthus*, *Hyacinthus*, *Ornithogalum* and *Iris germanica* normally do not form starch.

Brown and Morris from their study of the *Tropæolum* leaf were able to bring forward much evidence for the rejection of Sachs' view that all the carbohydrate formed in photosynthesis passes through the starch stage. The facts they adduce are more in accord with the idea put forward by Meyer in 1886 that starch is only produced when the supply of formative carbohydrates is in excess of the metabolic and translocating powers of the cell in which they are contained. Starch begins to be stored in the leaf when the concentration becomes too great for the normal requirements of the cell; the separation of the sugar as starch, that is in an insoluble form, relieves this concentration. The subsequent dissolution of the starch at night was shown by Brown and Morris to be brought about by an enzyme similar in its nature to the diastase of barley. All the plant leaves tested which form starch were shown to contain such an enzyme. Brown and Morris proved, moreover, that leaf diastase acts upon solid starch and that the amount of diastase in the leaves increases with the time that the leaf has been in darkness; this is not due to the fact that it accumulates as the starch disappears, owing to a diminished call upon it, but probably on account of diastase being produced as a starvation phenomenon. In accordance with the view that the starch is broken down by diastase, they give analyses showing the presence of maltose in oven-dried *Tropæolum* leaf. From similar material they isolated an osazone which, from an analysis and melting point, appeared to be identical with maltosazone. Maltose was regarded as a normal constituent of foliage leaves formed as a degradation product from the starch.

One of the most novel conclusions drawn by Brown and Morris was with regard to cane sugar. This was found to be present in even larger proportions than starch and the way in which it fluctuated in the leaf suggested that it was the first sugar formed in photosynthesis. Dextrose and laevulose were also present and were more readily accounted for as products of the hydrolysis of the cane sugar than as its precursors. Since laevulose was generally in excess of dextrose it was suggested that the latter is more quickly used up in respiration than the laevulose; therefore it seemed probable that under natural conditions a larger amount of laevulose than of dextrose passes out of the leaf into the stem in a given time.

In 1884 A. Girard[1] [1884] had also suggested that the saccharose which is stored in the root of the sugar beet is formed in the leaf of the plant by direct photosynthesis and transferred as such to the root. Girard's work, which from the quantitative standpoint was far more complete than anything previously published, was apparently overlooked by Brown and Morris in their 1893 paper. Girard's views were based on numerous analyses which showed the roots to contain saccharose only and no reducing sugar; reducing sugars, however, were present in the stalks (petioles) and leaf tissue. The roots and petioles were found to have the same composition by night as by day, but the proportion of saccharose in the leaves was much greater—frequently twice as great at the end of a day's insolation than next morning, after being several hours in darkness. The proportion of reducing sugars in the leaves, however, was sensibly the same in the evening as next morning and only increased as the plant developed. We shall discuss Girard's data more in detail later.

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[1] Girard gives a valuable summary of the investigations prior to 1884, dealing with the special problem of the formation and transmission of sugar in the sugar beet. In 1883 Kayser [1883] had found, by analysis, both cane sugar and reducing sugar in the

Since 1893 several papers have been published in which the formation of carbohydrates in the leaf is considered. These may be divided into two classes: (1) Those whose authors favour the view that saccharose is the first sugar formed in photosynthesis, (2) those in which the hexoses are regarded as primary products, the saccharose as formed later by synthesis either in the leaf or the root. We will briefly review these two classes separately.

1. *Cane sugar held to be formed directly.*

Went [1898] published observations on the distribution of reducing sugars and saccharose in the unripe sugar cane. The value of these is marred by the fact that, in the polarimetric estimation of cane sugar, Went calculated the reducing sugars as dextrose. Although Went held that cane sugar is the first formed sugar, he gives no experimental evidence in favour of this view. Strohmer [1908] relied upon the fact which was supposed to be established, first by Péligot and later by Girard [1884], that the roots of the sugar beet contain no sugar but saccharose; he states that, in the beet, reducing sugars never occur in the root, even in the early stages of growth. This, together with Girard's observation that in the night the saccharose content of the leaf fell to

leaves of several plants, e.g. *Beta vulgaris*, the grape vine (*Vitis vinifera*), potato, onion. In the early stages of growth, Kayser's analyses show that the cane sugar in the leaves is often greatly in excess of the hexoses, but later the proportion is increased. Kayser actually separated cane sugar from the leaves of the vine in a crystalline condition and with $[\alpha]_D = 62{\cdot}9°$.

one-half, is held to prove that the saccharose is formed in the leaf directly and migrates as such to the root in the night. He supports this view by observations on two abnormally formed beets in which the length of the neck had become enormously exaggerated; these abnormal growths contained cane sugar but no reducing sugar. If reducing sugars wandered from the leaf to the root they should have been found in large amount in the abnormally long necks. The objection to the view that the saccharose wanders as such, which is based on the supposed impermeability of the cell protoplasm to cane sugar, is, according to Strohmer, obviated if one assumes with Pfeffer that the protoplasm can change its properties periodically under the influence of a regulating mechanism. In a later paper [1911] Strohmer gives data for the second season's development of the sugar beet grown for seed, and shows that the total saccharose content of the root and principal stem is at the ripening period considerably greater than at flowering; the saccharose, however, during ripening entirely disappears from the leaves and stalks. This is held to confirm the view that the cane sugar is formed in the leaf and leaves it as such. Reducing sugars, however, predominate in the parts of the plant above ground at the flowering period because they have been formed by the inversion of saccharose, so as to be readily available for the building up of the flowers. Thus, for example, in the main stalk the ratio of reducing sugars to saccharose was 11·2 : 4·3. But later on, when ripening is near, the reducing sugar disappears almost completely.

Stephani [1911] also holds that saccharose is formed in the leaf of the beet and is transferred as such to the root for storage; the proportion of the reducing sugars in the root is generally very small (0·05 to 0·10 per cent.), but in some feeding varieties (*Fütterrüben*) may be as high as 0·5 per cent.

Parkin [1912] made a careful study of the sugars present in the leaf of the snowdrop (*Galanthus nivalis*), to which we shall refer more in detail later. He considers that his analyses strongly favour the view that saccharose is the first recognisable sugar to be formed in the leaf and that the hexoses arise from it through inversion. The rapid rise in cane sugar, which occurs in the leaf when it has been exposed to darkness for some days and is then again placed in sunshine, is particularly striking; thus in one experiment the cane sugar rose after 8 hours exposure to sunlight from 5 per cent. to 12·5 per cent., whilst the hexose changed only from 2·7 to 3·6 per cent. In darkness, on the other hand, it is the saccharose which falls rapidly whilst the hexoses remain nearly constant. Parkin's experiments were carried out with a plant, the snowdrop, which in normal growth never elaborates starch, so that complications which might arise from the presence of this carbohydrate or of maltose (which was shown to be absent throughout) were avoided. Parkin's view, which was also put forward by Brown and Morris in 1893, that the laevulose is present in excess of the glucose in the leaf—pointing to the latter contributing more readily to the needs of the leaf—is discussed in the next paper.

Peklo [1908], who studied the localisation of sugars in the beet by a microchemical method, concluded that the sieve-tubes of the phloem contain the greatest amount of cane sugar; he considers that the sieve-tubes serve mainly for the transit of the sugar and, after the formation of callus plates, for the storage of sugar in the root.

2. *Reducing sugars (hexoses) held to be the primary products and cane sugar to be formed from these.*

Maquenne [1895], in an attempt to explain the storage of saccharose in the beet, based on osmotic laws, stated that the osmotic pressure of the leaf sap is practically identical with that of the root sap. As it is essential to equilibrium that the concentration of the saccharose should be double that of the invert sugar, when, owing to photosynthesis, reducing sugars are formed in the leaf, they will travel to the root and there take up the form of saccharose.

Strakosch [1907] employing microchemical methods concluded that dextrose is formed in the mesophyll of the leaf of *Beta vulgaris* and is the only sugar found therein. The migration of dextrose into the leaf veins is followed by the appearance of laevulose in these, and later by the formation of saccharose. Strakosch considered that the cane sugar must be regarded as a final product in the leaf and migrates to the root as such. The amount of the monosaccharides in the leaf is not appreciably altered by the migration of the saccharose to the root, nor is it diminished when the leaves remain in the dark for some time. Exposure of the leaves to light does not cause the saccharose to increase beyond a certain maximum, which is attained in a short time.

In 1909 Robertson, Irvine and Dobson [1909] studied the distribution of enzymes in the roots, stalks and leaves of *Beta vulgaris*; they showed that invertase is present in the leaf and stem but absent from the root, and hence conclude that the cane sugar stored in the root is formed from antecedent monosaccharides by reversible zymohydrolysis in the leaf and stem and is thence translocated as such. The alternative view that the sugars travel downwards as hexoses, although meeting the diffusion difficulty, is not in accord with the absence of invertase in the root. Strohmer's analyses, which showed that practically no reducing sugars occur in the root, and Girard's conclusion that saccharose is present in all parts of the plant in the earliest stages of growth, also militate against this view.

Gutzeit [1911], on theoretical grounds based on the laws of diffusion and osmotic pressure, contended that monosaccharides are formed in the leaf and wander as such to the root where they are built up to cane sugar.

In the same year Ruhland [1911] contended that the sugar in the sugar beet does not pass from the leaf in the form of saccharose but as reducing sugars (perhaps largely as laevulose); the sugars entering the root consist mainly of reducing sugars which are resynthesised to saccharose within the root. The cells of the leaves and stalks are stated to be permeable to saccharose, raffinose, maltose and more or less to all the hexoses tested, from which they are able to form starch. Dextrose and laevulose are somewhat more diffusible than saccharose. Light does not appear to exercise any influence on the permeability of the cells, but on the other hand certain regulatory influences could be detected. Ruhland states that the young growing root contains invertase which gradually diminishes in quantity as the root matures and is confined only to the growing parts.

Deleano [1912], in a study of the respiration of the vine leaf (*Vitis vinifera*), found that only a small increase of reducing power was caused by inverting a solution obtained from the dried leaf material; this pointed to the presence of very small quantities of cane sugar relatively to the reducing sugars, which were present in large amounts, and as he failed to isolate any cane sugar by Schulze's strontia method he concluded that this sugar was in reality not present. We shall specially deal with this point later.

As distinct from the foregoing workers, who, on the one hand, regard saccharose as the first sugar formed in the leaf, and, on the other, consider the first product to be dextrose, Pellet [1913] considers that saccharose, dextrose and laevulose are formed simultaneously in the leaf and that the sugars descend in all three forms to the root, where the reducing sugars are built up to saccharose. In a later paper Pellet [1914, 1] shows that the view generally held, that the sugar cane contains no reducing sugars, is based upon early work which was carried out prior to the introduction of satisfactory methods of detecting or estimating reducing sugars. According to Pellet the reducing sugars which are found in the juice of the crushed cane are present as such within the cane itself and are not formed by inversion of the expressed juice. The proportion of reducing sugar to cane sugar increases on passing from the lower to the upper parts of the cane—that is nearer to the leaves; it grows less and less as ripening proceeds, pointing to a conversion of the reducing sugars into saccharose. Colin [1914] takes a similar view to Pellet. He shows that Girard's and Strohmer's assertions that reducing sugars are absent from the root of the beet are quite incorrect, especially in the early stages of growth, when the reducing sugar may form 20 per cent. of the total sugars. As the root develops and the store of cane sugar increases in it, the proportion of reducing sugar naturally falls, but it never entirely disappears and is always most abundant in the growing parts. In the neck the ratio of reducing sugar to cane sugar is highest. The root therefore receives at the same time both cane sugar and reducing sugars: the former is stored up and the latter polymerised to cane sugar. The entry of the sugars is regulated by the osmotic pressure of the mixture.

The recent work of Campbell [1911] and Kluyver [1914] will be dealt with later.

EXPERIMENTAL.

Methods of Work.

Destruction of enzymes. Much of the earlier work on the carbohydrates of the leaf is of doubtful value because insufficient care was taken to ensure that no change in the carbohydrates should occur after the picking of the leaves and during the preparation of the sample for analysis; changes brought about by enzymes are, as we shall show, very liable to occur either during the process of drying the leaf or during the expression of the sap, if a press be used. Whilst it is possible to express the juice from the root of the beet or mangold, or from the sugar cane, without much change in the cane sugar occurring, owing to enzymes being almost entirely absent in such cases, with leaf tissue such a process is unsafe, as enzymes are present in relatively large amount and are liberated from the cells in which they are contained by the mechanical pressure and thus have an opportunity to act upon the sugars during the time taken in preparing the sample. The work of Girard [1884] in particular, although he states that he worked as rapidly as possible, was liable to error from this cause; the leaves of the sample were first pulped in a special machine and then subjected to pressure so as to obtain the sap. Brown and Morris [1893], working with *Tropæolum* leaves, found in their preliminary experiments that considerable changes may occur in the sugars when the sample is prepared in this way, and Kluyver [1914] states that on estimating the cane sugar and reducing sugars in the sap of the same leaf he found practically no cane sugar, unless the juice were heated to 100° to destroy the enzymes before making the analysis. Brown and Morris in 1893, and Parkin [1912], and Kluyver more recently, therefore always attempted to destroy the enzymes in the leaves by quickly drying these in a steam oven. Parkin gives analyses which would indicate that in the case of the snowdrop this method of working brings about little change in the proportion of the sugars in the leaf. On the other hand, when the leaves are moderately thick, as in the mangold or sugar beet, and therefore heat up slowly, an opportunity is given to the enzymes to bring about considerable change in the carbohydrates before they are actually destroyed; enzyme action is especially likely to occur under these conditions, because the rate of action is greatly accelerated at first by the rising temperature. We shall show (see p. 357) that certain fundamental differences between our results and those of Brown and Morris and of Kluyver (for example, the entire absence of maltose in all the cases we have studied and our higher values for starch in the case of the *Tropæolum* leaf) are probably to be explained by enzyme action having taken place in the earlier experiments.

In all investigations hitherto, the material which was analysed was either pressed-out sap or an extract of the previously dried tissue. To ensure the destruction of the enzymes being as instantaneous as possible, we have adopted the following procedure. The freshly picked leaf material (about 1 kilogram) was dropped in small quantities at a time into a large volume (2 litres) of boiling alcohol, to which 1 per cent. by volume (20 cc.) of 0·880 ammonia was added so as to neutralise the acids present in the leaf; the quantity given is generally sufficient for this purpose. After the extraction with alcohol is complete, the solution is generally faintly alkaline to litmus paper; if this is not the case a little more ammonia should be added. By the method given it would appear that the enzymes in the plant tissue are instantly destroyed; the ammonia facilitates the destruction owing to its alkaline nature and its rapid diffusion into the plant cells. The nature of the results we have obtained gives us confidence that no changes occur in the leaf carbohydrates during this treatment or during the subsequent extraction of the sugars. Had any change, such as inversion, occurred, it would have been impossible to obtain, for example, the regular series of results actually found in the case of the potato leaf (see p. 367), where the cane sugar rises and falls between very narrow limits (2·1 to 3·5 per cent.) along curves which are practically straight lines.

Methods of Analysis. If work such as we have been engaged on is to have any permanent value, it is necessary to ensure that the analytical methods give trustworthy results with the class of material actually dealt with. Much time was therefore devoted in the beginning to testing these methods, and we have shown in several earlier papers (Davis and Daish [1913 and 1914]) that very grave errors may arise in investigations of this kind. Thus the important work of Brown and Morris in 1893, so far as it refers to the sugars, suffers from the fact that the only method then available to estimate maltose gave entirely incorrect results owing to the destruction of laevulose brought about by the hydrochloric acid used in the hydrolysis; as the accuracy of the values for the other reducing sugars, dextrose and laevulose, depends upon correct values being taken for the maltose, it is clear that the proportions found for these sugars are equally incorrect. We have shown also (Davis and Daish [1914]) that, in estimating starch by the diastase method, large errors may be caused by the loss of dextrin; we have introduced therefore a new method based on the use of taka-diastase, the enzyme of *Aspergillus oryzae*, which converts starch into a mixture of maltose and dextrose only. The method of hydrolysing cane sugar which was used by Campbell [1911] may, we have shown, give rise to entirely false results when applied to plant extracts, owing to the cane sugar being only partly inverted by the 2 per cent. citric acid which suffices to invert completely *pure* solutions of the sugar. In Campbell's analyses the cane sugar would therefore be underestimated; this would lead to very high values being returned for maltose, even though considerable destruction of laevulose occurred during the hydrolysis with hydrochloric acid. As the values for saccharose and maltose were incorrect, the data for dextrose and laevulose are equally invalid. Campbell's work on the carbohydrates of the mangold leaf must therefore be regarded as merely preliminary in a very difficult field and the data and conclusions entirely withdrawn.

Parkin's recent work [1912] was, fortunately, carried out with a plant in the leaves of which starch and maltose do not occur. The analyses were therefore not complicated by the necessity of estimating these substances. Parkin carefully tested many points of the analytical procedure used in estimating the cane sugar and reducing sugars; as will be seen later, many of our results confirm those obtained by Parkin (except as regards the dextrose : laevulose ratio, a subject which is dealt with separately, see Paper II), so that considerable confidence may be placed in these observations. The principal points open to criticism are: (1) How far the results were affected by the process of drying the leaves adopted by Parkin; (2) how far the cane sugar results were lowered owing to incomplete inversion occurring on account of the presence of lead acetate in the solution interfering with the ordinary Clerget process. From the experiments Parkin actually made to test these points it would appear that in the case of the snowdrop the error arising from either cause was but small.

In earlier papers (Davis and Daish [1913 and 1914]) we have given an outline of the methods of analysis we have adopted; we need therefore only add a few details which were formerly omitted.

Cane sugar has always been estimated by two distinct methods: by inversion with 10 per cent. citric acid [1913, p. 466] and by inversion with invertase (autolysed yeast). This gives a means of checking the results.

Maltose was estimated by the use of maltase-free yeasts [1913, p. 464], such as *S. marxianus* and *S. exiguus*, duplicate fermentations being carried out with ordinary baker's or distiller's yeast so as to make allowance for the *pentoses* present which remain unfermented.

Starch was estimated by taka-diastase [1914, p. 159] in the dry leaf material from which the sugars had been completely extracted by alcohol. Special details are given of this method, as applied to estimate "soluble starch" or "dextrin" when present, in our experiments on the potato leaf (see p. 361).

Pentoses. As shown in a previous paper (Davis and Sawyer [1914]) appreciable quantities of pentoses are invariably present in the alcoholic extracts of leaf material; these have been estimated by distilling a known volume (50 cc.) of the original purified solution used in the sugar estimations with hydrochloric acid and weighing the furfural produced as phloroglucide according to the Kröber-Tollens method.

Pentosans. A suitable quantity (1·5 grm. of the oven-dried leaf material, from which the sugars have been extracted, or about 1 grm. of extracted stalk) is distilled with hydrochloric acid under Kröber-Tollens conditions (Allen's *Commercial Organic Analysis*, I. 402); the furfural evolved is precipitated and weighed as phloroglucide.

For actual examples of the method of calculation see *Appendix*, pp. 315 to 319. All results are calculated as a percentage on the *total vacuum-dried matter* of the leaf (T.V.D.M.), that is on the sum of the alcohol-soluble and alcohol-insoluble substances.

Extraction of the sugars from the leaf material and preparation of the solution for analysis. The freshly plucked leaf material (about 1 kilogram) was cut off close at the end of the stalk and, after cutting out the mid-ribs, was dropped, in small quantities at a time, into two litres of boiling 95 per cent. alcohol to which 20 cc. of 0·880 ammonia had been added, contained in a large zinc beaker (14 ins. × 9 ins.) in which the alcohol could be safely boiled; after each addition, the whole mass was well stirred so as to immerse the newly added leaves and ensure the rapid destruction of the enzymes. The whole kilogram of leaf could be added in less than 10 minutes; the time of picking each sample was also about 10 minutes, the picking being commenced 5 minutes before the hour and completed 5 minutes after. As the Laboratory was near at hand, the whole mass of leaf could be added to the boiling alcohol in less than 30 minutes from the nominal time of picking. The stalks and mid-ribs were separately dropped into a smaller quantity of boiling alcohol (1 litre containing 10 cc. of 0·880 ammonia) contained in a smaller zinc vessel (12 ins. × 5 ins.). After the leaf or stalk material had been added to the alcohol, the latter was kept boiling about half-an-hour; the alcohol was then drawn off and transferred to the boiling vessel *B* of a large specially constructed zinc extraction apparatus, shown in Fig. 1[1]. The leaf material was packed into the extraction vessel *A*, which was fitted with a detachable false bottom of perforated zinc and acted on the principle of the ordinary Soxhlet extractor, the alcohol siphoning back from *A* into the boiling vessel *B* during the extraction. *B* was heated by a large water-bath. Two condensers were fitted at the top of the extractor to condense the alcohol vapour which was conveyed from the boiling vessel by a bent tube of compo-metal so arranged that it could be easily fitted to or disconnected from the apparatus, through the corks of which it passed. This tube and the extractor itself were wrapped in felt to minimise air-cooling. The extraction was generally complete after about 12–18 hours; preliminary experiments showed that the whole of the sugars are removed when the leaf becomes colourless. To hasten the extraction, the leaves should be turned out of the extractor after about 10 hours and the portions which were at first at the bottom (and therefore less completely extracted and still green in colour) put back at the top, where the alcohol is hotter and acts more efficiently. In carrying out a series of pickings every 2 hours over a period of 24 hours, we have used four large extractors, the later samples being put into the apparatus as soon as the earlier samples were completely extracted. Three smaller apparatus served to deal with stalks and mid-ribs.

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[1] For the mid-ribs and stalks a smaller extraction apparatus of zinc was used. The dimensions of vessel *A* were 7″ × 4″, and of *B* 5″ × 5″, with a 2″ neck.

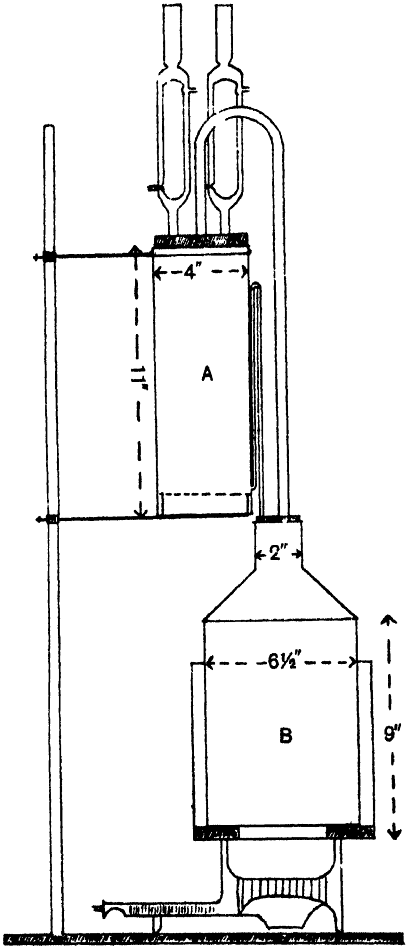

Fig. 1. Extraction apparatus. ⅛ size.

When extraction is complete (as judged by the leaves being practically colourless) the alcohol in the extractor is allowed to siphon over into *B* as completely as possible and the leaves are transferred to a small jute bag[1]; the alcohol is then expressed by means of a Buchner hydraulic press. It is generally quite colourless and is added to the extract in *B*. The leaf material remaining is obtained, after the pressing, in a practically dry condition as a hard cake; it is shredded apart and the loose material obtained dried on paper trays in a steam oven for 18 hours; the mass obtained is then weighed on a rough balance (to the nearest centigram), quickly ground in a small mill, and left in an air-tight bottle until it can be analysed. In this material the *moisture* lost by drying at 110° *in vacuo*, the *starch* and *pentosans* are subsequently determined.

The alcoholic extract, which with washings, amounts usually to nearly 3 litres, if it cannot be immediately analysed (as is usual in a series of extractions) is transferred to a large bottle, about 10-20 cc. of toluene is added and the bottle closed with a paraffin-waxed cork. We have found, in preliminary experiments, that alcoholic extracts made in this way can be stored for 3 to 6 months without the slightest change, even inversion of the cane sugar, occurring. Care should be taken that the solutions are practically neutral or only *very* faintly alkaline to litmus paper; this is usually the case when ammonia has been added in the proportion stated above, but if any acidity can be detected in the solution it should be corrected by adding the proper quantity of ammonia so as to make the solution *just* alkaline to litmus.

The alcoholic extract serves to estimate the *total soluble matter* and the *sugars* of the leaf (saccharose, maltose, dextrose, laevulose and pentoses). For this purpose it is evaporated *in vacuo* (20-30 mm.) in the special apparatus devised for this work (Davis [1913]) which needs practically no attention and enables the 3 litres of extract to be reduced to a small volume (100-150 cc.) in a few hours at a temperature not exceeding 35-40°. At so low a temperature all possibility of change in the sugars is obviated. When the extract has been reduced to 150 cc. it is transferred to a 500 cc. measuring flask[2]. When much chlorophyll or fatty matter is present it is necessary to wash out the flask with a little hot alcohol or toluene; in this way there is no difficulty in transferring to the measuring flask every trace of soluble matter, whether sugars or leaf fats. The solution is then diluted to 500 cc. at 15° and is usually fairly homogeneous; when, however, toluene has been added the mixture should be thoroughly shaken so as to form a fine emulsion immediately before withdrawing each of the samples for the dry matter estimations. Working in this way the results are usually quite concordant, the differences seldom exceeding 0·2 per cent. on the weight of dry matter.

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[1] This should always be boiled with water several times before use to extract any soluble substances (dextrin, etc.).

[2] In the case of stalks and mid-ribs the alcoholic extract (usually about 1500 cc.) is evaporated *in vacuo* to about 25-30 cc.: it is then transferred, with washings, to a 100 cc. flask and made exactly to volume. Three portions, each of 10 cc., are used for the estimation of *dry matter* and the remaining 70 cc. after precipitation with basic lead acetate (usually 70-100 cc. are required) are filtered off on a Buchner funnel, and washed until the volume of the filtrate is nearly 500 cc. The excess of lead is removed by adding exactly the necessary quantity of solid sodium carbonate and the solution diluted to 500 cc. 25 cc. portions of the clear filtered solution are used to measure the *direct reducing* and

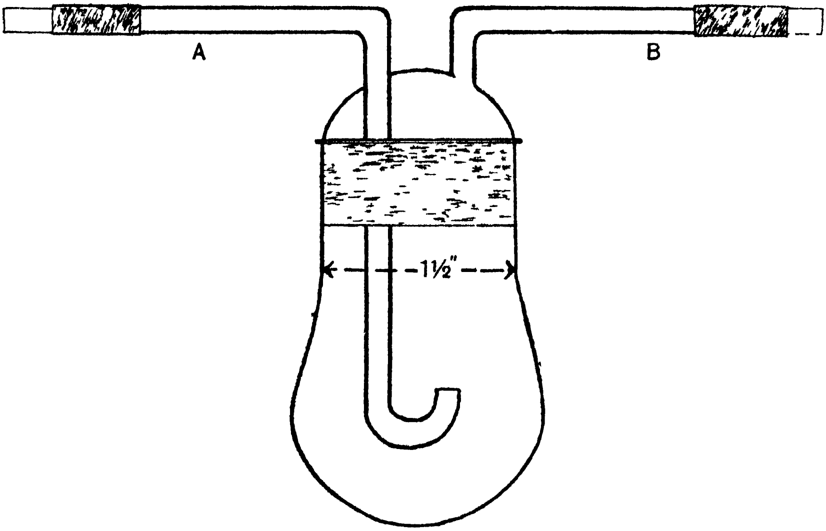

Fig. 2 Flask used to estimate dry matter in the alcoholic extract.

For the *dry matter estimations*, 20 cc. of the 500 cc. are transferred by means of a pipette to the small flask shown in Fig. 2, evaporated to dryness on a water-bath and finally dried *in vacuo* at 110°; three samples of 20 cc. should be withdrawn, two being used for the actual estimations and the third kept as a reserve in case the duplicates do not agree sufficiently closely. Usually the duplicates agree, when say 1·5 to

rotatory powers and portions of 50 cc. for inversion with citric acid and with invertase. After inversion these portions are neutralised, diluted to 100 cc at 15° and 50 cc. of the solutions so obtained are used to measure the increase of reducing power or change of rotatory power caused by inversion. This 50 cc. corresponds with the 25 cc. used for the *direct* measurements. 50 cc. of the 500 cc. are used to estimate the *pentoses*, and 50 cc. portions are fermented with the special maltase-free yeasts to estimate *maltose*. controls being also made with baker's yeast.

2·0 grms. of dry matter are weighed, to within a few milligrams, so that the error involved in this method is considerably less than the probable error of sampling. The apparatus shown in Fig. 2 consists of a small *round*-bottomed flask with a light, ground-in glass stopper which carries two side tubes as shown, one of which, *A*, passes to within an inch of the bottom, whilst the other, *B*, only just enters the stopper. The two tubes can be closed with glass plugs as shown. The flask and stoppers are first weighed alone and then the 20 cc. of extract is evaporated in the flask as far as this is possible in the water-bath; the stopper is then inserted and the flask placed in a copper Meyer bath, filled with toluene, the end of the tube *A* being connected with the vacuum pump, a flask of phosphorus pentoxide being interposed to absorb moisture. An ordinary Meyer bath serves to heat two of these flasks at the same time, the copper lid of the bath being replaced by an asbestos cover with two holes cut in it to take the necks of the flasks. In all cases the dry matter was heated 18 hours *in vacuo* at 110°, the weight being then practically constant. Unless the drying be carried out *in vacuo* in the manner described it is impossible to obtain anything like constant results.

The apparatus shown in Fig. 2 serves also to estimate the ***moisture*** in the leaf matter left after extracting the sugars (see p. 268) prior to the estimation of *starch.*

Estimation of sugars. The 440 cc. remaining of the original 500 cc. are transferred to a large flask, diluted with about 300 cc. of water and *exactly* the necessary quantity of basic lead acetate[1] added to precipitate the whole of the tannins, amino-acids, etc. present; care should be taken to avoid any considerable excess of the basic lead, which must be added in small quantities at a time, until on filtering a small portion of the solution and testing it no further precipitate is produced. Working in this way it is possible to avoid having more than 1 or 2 cc. of the lead acetate solution in excess at the end of the precipitation. The quantity of lead acetate solution used[2] with different samples varies widely, according to the nature of the leaf, the time of year, etc.; when 1 kilogram of mangold leaf is used, the 440 cc. of the sugar solution requires from 200–300 cc. After the precipitation is complete the solution is filtered on a large Buchner funnel (6 ins.) and the precipitate pressed down and washed until the filtrate and washings have a volume of nearly 2 litres. A little solid sodium carbonate is then added[1] until the lead is *exactly* precipitated, testing small portions so as to avoid any considerable excess of sodium carbonate; the solution is then diluted to 2000 cc. and a little toluene added (1 cc.) to obviate bacterial or fermentative change. We have found that a solution prepared in this way can be left for several weeks without showing any change in the proportion of sugars present; but care must be taken that the solution is not left for any length of time, even a few hours, with *any excess of basic lead acetate* or any *considerable quantity of alkali, as both of these substances rapidly destroy laevulose.*

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[1] We show in a separate paper that, by using basic lead acetate in the manner we prescribe, no loss or destruction of the sugars is to be feared.

[2] We use the ordinary solution of basic lead acetate as employed in general sugar analysis (sp. gr. 1·25, see Allen's *Commercial Organic Analysis*, vol. I. p. 308).

The actual estimation of the sugars is described in a previous paper [1913, p. 466].

Polarimetric Arrangements.

With plant extracts such as those we have been studying the purified solution finally used for analysis is very dilute and therefore gives very small angular readings in the polarimeter—generally less than 1° in a 200 mm. tube. For the results to be correct within 1 per cent., the angular readings must therefore be accurate to within 0·01°. As slight differences in temperature cause considerable alterations in the specific rotatory power of laevulose and invert sugar, precautions must be taken to ensure constancy of temperature during the observations. All our readings have been taken exactly at 20·00°. By means of the following simple thermostatic arrangement it is easy to maintain this temperature to within $\frac{1}{100}$° for weeks together (Fig. 3).

The temperature of the bath *W*, which consists of a large enamelled iron vessel 16 ins. × 10 ins., is controlled by a Lowry toluene thermo-regulator *A*; the stirrer *B* is constructed from an old bicycle hub by lengthening the spindle in both directions and attaching above a wooden pulley 7 ins. in diameter, and below a four-bladed paddle. This stirrer and the small Köhler centrifugal pump *C* are run from the same small electric motor ($\frac{1}{80}$ h.p.) by a three-grooved pulley. The water-bath *W* is kept covered by a tin-plate cover, with holes and grooves cut in it to admit the neck of the thermo-regulator and other fixtures; a thermometer (not shown in drawing) graduated in hundredths of a degree also passes through this cover and enables the temperature to be read accurately to $\frac{1}{200}$° C The water of the bath, which is maintained at a constant temperature by the thermo-regulator, is circulated through the jacket of the polarimeter tube and back to the bath by means of the small centrifugal pump *C*; it takes only about 5 minutes to bring the temperature of the tube exactly to 20·0°.

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[1] When the content of sugars is too high to allow of the analysis being made directly by Brown, Morris and Millar's method, 300 cc. of the 2 litres should be diluted to 500 cc.

The polarimeter we have used is one of Schmidt and Haensch's Lippich instruments, with a triple field, capable of taking a 400 mm. tube and reading in angular degrees to 0·01°; it is fitted with a spectroscopic attachment and a scale enabling the instrument to be used with mono-chromatic light of any desired wave-length. All the observations recorded in the present paper were made with a sodium lamp, but for this class of work a mercury vapour lamp is preferable as it obviates the need of constantly replenishing the flame, gives a far brighter light and is much cleaner in use. We have used very successfully a Lummer-Straubel *glass* lamp, fitted with a water cooling jacket, which is preferable to the quartz mercury vapour lamps as it gives no trouble with ultra-violet rays, is far cheaper and throws a small concentrated light directly into the polarimeter tube. This lamp needs 25 volts, but can be run from an ordinary 100 volt circuit by interposing a suitable resistance; for this purpose we have used a wall type adjustable resistance with a radial switch controlling twelve contacts giving a range of 2–10 amps.

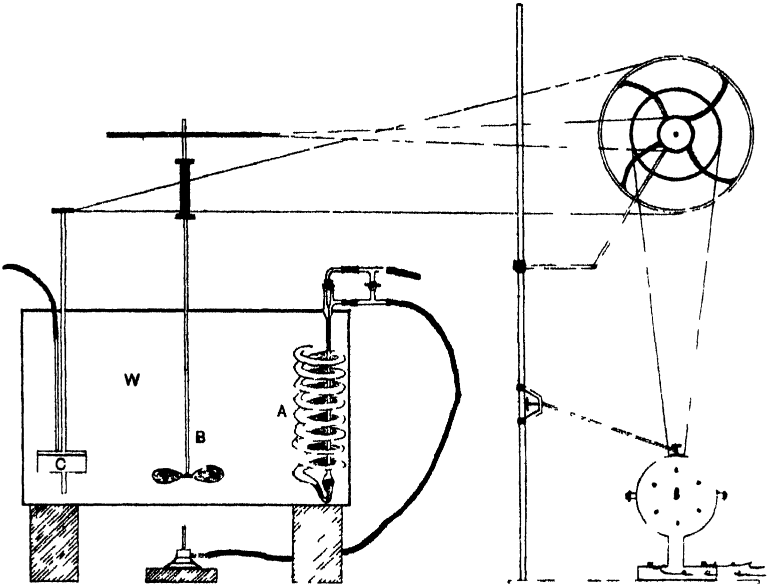

Fig. 3. Thermostat for polarimetric measurements.

at 90 volts drop, the last three contacts giving 10 amps. at 80, 70 and 60 volts drop. This was made for us by Messrs Tyler and Freeman, London.

Experience has shown that by taking eight to ten readings it is possible with our instrument to obtain values which have a probable error considerably less than ± 0·005°. Duplicate sets of readings with a clear solution generally agree to within 0·002°.

Probable error of the Analyses and Methods of Sampling.

A. *Methods of analysis.* To ascertain whether any inversion of cane sugar is caused during the evaporation *in vacuo* and the subsequent treatment of the alcoholic extract, and also the degree of accuracy with which cane sugar can be estimated in such solutions, the original extract from a picking of mangold leaves having a volume of about 3 litres was made up exactly to 4 litres. It was then divided into two halves, each exactly of 2 litres, and to one of the halves 5·00 grms. of pure saccharose was added, to the other nothing. Each half was then evaporated *in vacuo* as usual and subjected to the ordinary processes of analysis. As a result it was found that the difference between the average values in the two cases, *by the reduction process*, using both the invertase and citric acid methods of inversion, corresponded with 4·98 grms. instead of the 5·00 grms. actually added. The *increase* of polarisation in the direct solution due to the added sugar gave a slightly higher value, 5·22 grms. saccharose, whilst the results calculated from the *change of polarisation on inversion* gave for the added sugar:

$$\left.\begin{array}{ll} 1.\ \text{Invertase} & 5{\cdot}80 \\ 2.\ \text{Citric acid} & 6{\cdot}16 \end{array}\right\}\ \text{average} = 5{\cdot}98\ \text{grms.}$$

The results obtained by the *change of polarisation* on inversion are therefore *nearly* 20 *per cent. high.* Throughout our work we have observed a similar divergence between the results obtained by the reduction and polarisation methods; the explanation of this is given later (see p. 329). Consequently in discussing the variation of the sugars throughout the day we have used the reduction values only and ignored the polarisation results, which are undoubtedly high in the majority of cases and especially so in the case of the stalks and mid-ribs. The value 4·98 obtained for saccharose by the reduction method as compared with the 5·00 grms. actually added shows that no inversion or loss of cane sugar is to be feared under our conditions of working and that the reduction method is quite trustworthy.

B. *Error of sampling.* The leaves of the mangold on different plants at any particular date are very variable in size so that some doubt might be felt as to the range of variation of the sugars due to differences of sampling. Our practice has been to pick about 120 medium-sized leaves from the plot dealt with, ignoring the very large and very small leaves. In all cases care was taken to choose leaves of a good colour and normal growth, well exposed to light; one leaf only has been taken from each root at each picking. To obtain an idea of the probable extreme error of sampling under these conditions, two lots of leaves were picked at precisely the same time (2.45 p.m., October 8th, 1914) from the same plot, at a late stage of growth when the difference between individual plants is at its greatest. These, on analysis, gave the following results:

	From reduction values		Cane sugar % from change of polarisation on inversion	
	Hexoses calculated as invert sugar % on T.V.D.M	Saccharose % by inversion on T.V.D.M.	Direct solution read when faintly alkaline	Direct solution read in presence of SO_2
First sample ...	19·0	7·52	—	8·62
Second sample	17·9	7·57	8·09	8·35

Whilst there is a very close agreement (7·52 and 7·57 per cent.) for the two samples in the case of cane sugar estimated by the change of reducing power on inversion, there is a considerably greater difference between the values for reducing sugars, calculated as invert sugar; the difference is about a single unit or about 6 per cent. of the total reducing sugars. The values obtained for saccharose from the change of rotation on inversion are, as is usually the case, from 7 to 15 per cent. higher than the reduction values; no closer agreement is obtained by taking the initial direct reading after saturating the solution with sulphur dioxide so as to have it acid and not faintly alkaline, in fact the difference is slightly *greater*. The cause of this difference is dealt with in the next paper.

The above case probably gives an extreme value for the error of sampling, as owing to the very dry autumn the leaves of most of the plants were beginning to turn yellow and were far more variable than is usual at this time of year, so that it was difficult to get a fair and representative sample. The mangolds were lifted about three weeks earlier than usual and this picking was made four days before the lifting of the roots began. In ordinary cases, the error of sampling in the case of the saccharose estimations is probably negligible, and in the case of the reducing sugars not greater than 2 or 3 per cent. of the actual values.

RESULTS OF MANGOLD EXPERIMENTS.

The mangolds used were Sutton's Yellow Globe and were grown on Plot 9 of Barn Field[1], which is manured with minerals and nitrate of soda. The pickings were taken so as to obtain information as to the sugars in the leaves and stalks at different stages of growth. During the early period of growth the plant is mainly occupied in forming leaf, and the root is relatively small, consisting merely of a tap root and root hairs; later on the leaf formation reaches a maximum and the root then develops rapidly and stores sugar abundantly, little increase taking place in the leaves. Records of the ratio of leaf to root during growth in recent seasons are not available for the mangold, but the systematic experiments on the closely allied sugar beet carried out in France and Germany in 1913 and recorded by Vivien [1913] show that from June 17th to August 26th, whilst the leaves steadily increased in weight, the ratio of the weight of leaves to root fell from 6·06 to 1·61; during the next month, August 26th to September 30th, when growth was finally complete, the weight of the leaves was actually falling, but the root increased by large amounts. The ratio of leaves to root fell in this period from 1·61 to 1·04. When the roots are lifted the weight of the leaves is nearly the same as the weight of the roots. In the case of the mangold, which stores a much smaller proportion of sugar, the weight of leaves at lifting is always much smaller than the weight of the root—generally only about one-third to one-quarter, as shown by the data given in the note below and the records of the Rothamsted experiments. The relationship of the leaf to root in the sugar beet and mangold is in accord with the fact that in the beet the percentage of sugar is roughly three to four times that of the mangold (yellow globe), so that the ratio of leaf to root and the percentage of sugar are roughly proportional values.

Our samples were taken at three different dates, so as to give data representing three distinct stages of growth:

Series I. Early growth, when leaf formation predominates. Samples were taken every two hours during a complete period of 24 hours.

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[1] This plot receives as manure 500 lbs. of potassium sulphate, 200 lbs. of magnesium sulphate, 200 lbs. of sodium chloride and 550 lbs. of sodium nitrate to the acre. The yield was on this plot in 1912: roots 17·95 tons, leaves 6·08 tons per acre; in 1913, roots 21·2, leaves 7·06 tons per acre.

starting at 6 a.m. on August 26th and ending at 4 a.m. next morning (August 27th, 1913). The seeds were sown for this crop on June 9th.

Series II. Intermediate growth, September 10th–11th, 1912, when leaf formation is relatively small and the sugars are being vigorously stored in the root, which is growing rapidly.

Series III. Final stage of growth, October 11th–12th, 1912; growth of root practically complete, roots lifted at end of October.

Method of expressing the results.

As the amount of water in the leaves and stems varies widely with the meteorological conditions, the method of analysis described above was decided upon, so that the results could be calculated upon the *total vacuum dried matter* of the material dealt with. From the data obtained it is possible also to calculate the relationship between the material soluble in alcohol and that left undissolved and also the ratio existing between the sugars at the different times of picking.

A. THE SUGARS OF THE MANGOLD LEAF.

Series I. Early Growth, August 26th–27th, 1913.

The results obtained in the first series of pickings (August 26th–27th, 1913) are given in Table I, and are shown graphically in Fig. 4.

Maltose and *Starch* are entirely absent from the mangold leaf throughout the day and night. This is true also of the later stages of growth[1] (see Tables II and III).

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[1] We have found that although very young seedlings of the mangold store starch abundantly in the leaf, the starch disappears entirely as soon as the root begins to grow and becomes capable of storing the sugars elaborated in the leaf. It would therefore appear that the mangold has the power of forming starch but never exercises it in the later stages of growth when the sugars formed in the leaf can readily be translocated away. The leaves of very young plants appear, when examined by the chloral-hydrate-iodine method, to be gorged with starch after a bright day, probably owing to the fact that the formation of starch at this stage is of service in preventing too high a concentration of the sugars in the leaf cells which cannot be dealt with by other methods, but after about the end of July, as our analyses and microscopic tests have shown, starch is invariably absent because the sugar can then be translocated to the root and prevented from accumulating. These facts throw a clear light on the function of the formation of starch in the leaf, which clearly serves to reduce the concentration of the sugars and thus prevent it from attaining too high a value, such as would be prejudicial to the plant. In this connection the fact established in 1885 by A. Meyer is of importance, viz. that plants which store starch abundantly contain comparatively little of the reducing or non-reducing sugars, whilst leaves of plants like *Iris germanica*, *Allium cepa* and snowdrop, which form very

Saccharose and Reducing Sugars. The data given in Table I show that, on the whole, there is a close agreement between the values obtained for cane sugar by the two methods used—inversion by citric acid and inversion by invertase. The values given are those obtained by the reduction method; the results obtained by the double polarisation method, as pointed out on p. 274, are uniformly higher. The cause of this difference and of the fact that the reduction values obtained by citric acid are nearly always slightly, but only slightly, higher than the corresponding invertase figures, will be discussed in a separate paper.

Fig. 4 shows that the cane sugar and hexoses both begin to increase in amount immediately after sunrise; the increase follows more or less closely the temperature curve[1]. But whilst for the two sets of sugars the increase from 6 a.m. to 10 a.m. takes place practically along straight lines, this is not the case with the temperature, and the maximum of reducing sugar is reached at 10 a.m., considerably before the maximum either of temperature or cane sugar; the maxima of these last two curves however synchronise at about 2 p.m. During the period of daylight the cane sugar curve is roughly parallel to the temperature curve, the saccharose rising as the temperature rises and falling as the temperature falls. On the other hand, the hexoses, which at first increase more rapidly than the saccharose, subsequently, between 10 a.m. and 4 p.m., fall more abruptly than this sugar and during the night period follow almost a straight line, which is very nearly parallel to the straight line which shows the fall of the cane sugar.

The important thing to be noted with regard to these curves is their comparative simplicity as compared with the corresponding curves later in the season (see Figs. 5 and 6). No night maximum is observed

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little starch in the leaf, show high concentrations of sugars (compare Parkin [1912]). As we show later, the potato, which forms starch abundantly, only contains a small proportion of sugars in the leaf.

The dependence of the starch content of the mangold leaf on the degree of development is well shown by the results obtained on examining leaves of the mangold which were plucked simultaneously on a bright day, at 11 a.m., July 15th, 1915, from plants growing on differently manured plots. Leaves from plots 5 O, 6 O, 8 O, which lack nitrogenous manure, were very small, and contained an abundance of starch; in all these cases the root was still very small. On plot 2 N (dung, super, potash and sodium nitrate) the leaves were much larger (3 ins. × 2 ins.), but still showed some starch; but on 2 A, where the leaves were much farther advanced (5 ins. × 2½ ins.) starch was practically absent. Even the guard-cells of the stomata were nearly empty.

[1] We do not wish to infer that the increase is merely a temperature effect: the rise of the sugars is probably directly related to the intensity of solar radiation and is a photochemical effect of which the temperature gives merely a rough index.

such as occurs later on, although the percentage of cane sugar actually found at 12 midnight is distinctly above the straight line drawn to indicate the falling off of the saccharose; a fact which suggests that a slight increase occurs in this sugar, similar in its nature but on a

TABLE I.

August 26th. Sun rises 5.4 a.m. Sun sets 7.0 p.m. August 27th. Sun rises 5.5 a.m. Sun sets 6.59 p.m.

Time	Temp.*	% of leaf		Sacc % in T.V.D.M.		Δ = C A. − I.	Saccharose av. %	Hexoses % as I.S.‡	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I.S./C S. (av.)	Remarks
		Soluble† in alcohol	Insoluble in alcohol	Citric acid	Invertase										
6 a.m.	45° F.	42·5	57·5	2·56	2·47	+0·09	2·51	0·77	3·28	0·37	5·38	0·00	0·00	0·307	Mists clearing away
8 a.m.	62°	42·5	57·5	2·76	2·75	+0·01	2·75	1·42	4·17	0·42	5·33	,,	,,	0·517	Brilliant sunshine
10 a.m.	70°	40·8	59·2	3·02	3·05	−0·03	3·04	2·16	5·20	0·44	5·65	,,	,,	0·710	,, ,,
12 noon	73°	40·6	59·4	3·17	3·04	+0·13	3·11	2·15	5·26	0·41	5·53	,,	,,	0·691	,, ,,
2 p.m.	75° (max)	37·3	62·7	3·23	2·96	+0·27	3·09	1·94	5·03	0·41	5·61	,,	,,	0·628	Bright sun
4 p m.	71°	37·2	62·8	3·09	3·01	+0·08	3·05	1·18	4·23	0·41	5·39	,,	,,	0·387	Still quite bright, but slightly hazy
6 p.m.	66°	40·1	59·9	2·96	2·68	+0·28	2·82	0·96	3·78	0·45	5·19	,,	,,	0·341	Bright sunshine
8 p.m.	61°	37·1	62·9	2·47	2·22	+0·25	2·35	0·90	3·25	0·36	5·50	,,	,,	0·383	Just dark
10 p.m.	58·5°	40·4	59·6	2·22	2·09	+0·13	2·15	0·74	2·89	0·37	5·31	,,	,,	0·344	Dry
12 midnight	58°	38·0	62·0	2·41	1·96	+0·45	2·18	0·57	2·75	0·42	5·65	,,	,,	0·261	Dry, but cloudy
2 a.m.	57°	38·6	61·4	1·70	1·46	+0·24	1·58	0·38	1·96	0·40	5·64	,,	,,	0·241	Cloudy
4 a.m.	56° (Min. 54° at 5 a.m.)	38·0	62·0	1·71	1·28	+0·43	1·50	0·20	1·70	0·52	5·96	,,	,,	0·133	Slight rainfall at 4 a.m.
6 a.m.	54·5°	—	—	—	—	—	—	—	—	—	—	—	—	—	First dawn = 4.45 a.m.
8 a.m.	57°	—	—	—	—	—	—	—	—	—	—	—	—	—	

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* The temperatures given are the temperatures of the air recorded by the shaded automatic-recording instrument near Barn Field, 5 ft. above the ground.

† These values give the percentage of vacuum-dried solids of the leaf which are soluble in alcohol, the percentage being calculated on the *total* vacuum-dried matter of the leaf.

‡ The reducing sugars are here calculated as invert sugar after allowing for the pentoses present. If the separate amounts of dextrose and laevulose are calculated (see p. 318) the *sum* of the results, as a rule, differs only very slightly from the values given here. The question of the dextrose-laevulose ratio is discussed in a separate paper (see p. 327).

smaller scale than the increase which is found at this time of night in the later stages of growth. No stress can, however, be laid upon

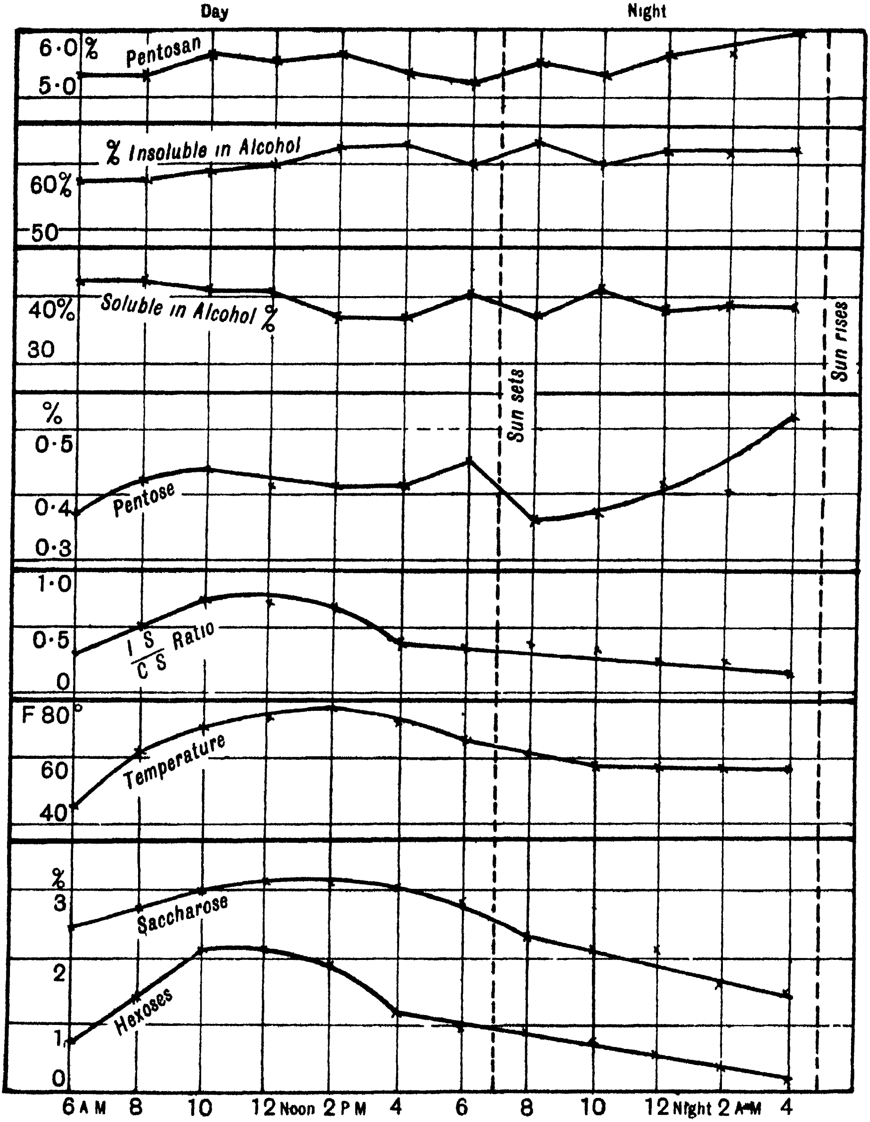

Fig. 4. Mangold leaf, Series I, Aug 26–27, 1913

this slight departure from a regular falling off in the sugar, as it might well be occasioned by error of analysis or sampling. That this error is, however, in general quite small may be inferred from the regular course pursued by the curves in question. The total variation of the cane sugar during the day is only from 2·5 to 3·1 per cent., but the methods of working adopted are sufficiently delicate to show a regular and progressive variation between these extremes.

The outstanding features of the sugar curves are:

1. The rise of sugar which first occurs, followed by a rapid falling off along approximately straight lines. Practically the whole of the reducing sugar disappears during the night, but the saccharose only falls to about one-half of its maximum value (3·1 per cent. falls to 1·5 per cent.).

2. The quantity of saccharose is always greater than that of hexose sugar (seven times as great at 4 a.m., 1·5 times as great at 10 a.m.), so that the saccharose curve is always well above the hexose curve. But the hexoses increase at first more rapidly than the saccharose and later on fall off more quickly. The curve showing the ratio of hexoses to saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}}\text{ ratio}\right)$ is itself more or less closely parallel to the temperature curve, a fact which becomes even more strikingly marked in Series II. Thus the proportion between the sugars—the increase of the hexoses relatively to the saccharose—seems itself to be a function of the temperature, or perhaps of the photo-chemical activity of which the temperature is in this case a rough measure. We shall discuss this point later (see p. 312).

3. The total fluctuations of the sugars are very small, especially in comparison with those found later in the season.

The saccharose increases from 2·5 per cent. to 3·11 per cent. and falls at night to 1·50 per cent.

The hexoses increase from 0·77 per cent. to 2·16 per cent. and then fall at night to 0·20 per cent.

The fluctuation of the hexoses is far greater than of the saccharose.

Pentosan Curve and Curve of Matter Insoluble in Alcohol.

From Fig. 4 it is seen that in spite of the considerable *increase* in the sugars which occurs during the day from 6 a.m. to 2 p.m., there is simultaneously a marked increase in the percentage of matter *insoluble* in alcohol; this increase runs closely parallel with an increase in the *pentosans*. The curve of insoluble matter in fact closely resembles the pentosan curve, a resemblance which becomes far more strongly marked in the September picking (see Fig. 5, p. 283), when the curves are practically identical in form. In Fig. 4 the rise and fall of matter insoluble in alcohol which occurs just before and after dark (6 p.m. to 10 p.m.) is accompanied by a corresponding rise and fall of pentosan; the increase of pentosan at this point seems to be associated with the sudden falling off of free pentoses, which occurs between 6 and 8 p.m., but the pentoses subsequently rise during the night, side by side with the rise of pentosans and of matter insoluble in alcohol.

The increase in the amount of pentosan and of matter insoluble in alcohol which is visible during the day in spite of the increase in the amount of substances soluble in alcohol is partly due to the formation of new ligneous tissue, but is probably more the result of the formation of gummy substances, which we have found always to be present in considerable quantity in the leaf tissue. These gums, as we shall show later, probably play the part of reserve substances (see p. 285).

Although the pentosan does not vary within very wide limits (5·2 to 5·96 per cent.), the value at the end of the 24 hours (5·96) is considerably higher than at the commencement (5·38); a similar but even larger increase is found at the September picking (see Table II and Fig. 5). This is probably due to the increase of the ligneous constituents of the leaf. In October when the pentosan has increased to about 7 per cent., and the leaves are no longer growing, there is very little variation during the day (6·89 to 7·15) other than can be accounted for by the much wider variations of the total sugars.

Pentoses. Between 6 a.m. and 4 p.m. there is a slight rise in the pentoses, followed by a slight fall, the curve running more or less parallel with the other sugar curves. Between 4 p.m. and 8 p.m. there is an abrupt rise of the pentose followed by an abrupt fall, these changes synchronising with a fall and a rise respectively of pentosan. During the night the pentose rises fairly steadily, and the same is true of the pentosan, both changes apparently taking place at the expense of the saccharose and reducing sugars, which are falling steadily throughout the night—especially the hexoses, which practically disappear. These facts and the parallelism of the pentose curves with those of the other sugars during the day, suggest that the pentoses arise from the reducing sugars and the pentosans from the pentoses.

At this stage of growth, when leaf formation is predominant, it is interesting to note that the pentoses (0·41 per cent.) at their midday maximum have roughly the same ratio to the pentosan tissue (5·5 per cent.) as the other sugars (5·2 per cent.) have to the total insoluble leaf material (60 per cent.), the ratio being roughly $\frac{1}{12}$.

The total fluctuation during the 24 hours is small—ranging only from 0·37 to 0·52 per cent.

Series II. Intermediate Stage of Growth.

TABLE II.

Mangold Leaves, September 10th–11th, 1912.

Sept. 10th. Sun rises 5.28 a.m. Sept. 11th. Sun rises 5.30 a.m.
Sun sets 6.26 p.m. Sun sets 6.24 p.m.

Time	Temp.	% of leaf*		Sacc % in T.V.D.M.		$\Delta = \text{C A} - \text{I}$.	Saccharose av. %	Hexoses % as I S.†	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I S / C S (av)	Remarks
		Soluble in alcohol	Insoluble in alcohol	Citric acid	Invertase										
10 a.m.	50° F.	54·7	45·3	4·60	4·43	+0·17	4·51	5·72	10·23	0·34	4·42	0·00	0·00	1·27	Dull, cold wind
1 p.m.	53°	44·2	55·8	4·86	4·57	+0·29	4·62	7·50	12·12	0·39	5·74	,,	,,	1·59	Dull, cold wind
4 p.m.	50°	48·5	51·5	4·41	4·35	+0·06	4·38	7·00	11·38	0·68	5·25	,,	,,	1·60	Dull, slight rain 3.30
6 p.m.	49°	51·0	49·0	6·46	6·32	+0·14	6·39	8·90	15·29	0·45	5·18	,,	,,	1·39	Dull. slight rain
Dark 7 p.m.															
8 p.m.	47°	47·2	52·8	5·61	5·27	+0·34	5·44	6·76	12·20	0·71	5·52	,,	,,	1·24	Clear, starlight
11 p.m.	43°	49·8	50·2	6·35	6·47	−0·12	6·41	7·10	13·51	0·71	5·31	,,	,,	1·11	,, ,,
2 a.m.	44°	50·7	49·3	8·28	8·26	+0·02	8·27	7·81	16·08	0·76	5·29	,,	,,	0·94	Cloudy
4 a.m.	44°	51·3	48·7	5·68	5·57	+0·11	5·62	6·91	12·53	0·62	5·26	,,	,,	1·23	Cloudy
Light 5 a.m.															
6 a.m.	44°	47·5	52·5	4·23	4·24	−0·01	4·24	6·30	10·54	0·65	5·67	,,	,,	1·48	Overcast
8 a.m.	46°	45·7	54·3	4·79	4·42	+0·37	4·60	5·38	9·98	0·61	5·90	,,	,,	1·17	,,

* Calculated on total vacuum-dried matter of the leaf.

† After allowing for the pentoses present.

Changes during the 24 *hours.*

The results are shown graphically in Fig. 5.

Maltose and Starch. These are absent throughout the whole 24 hours.

Saccharose and Hexoses. As in Series I the results of the inversion with citric acid are slightly higher than those obtained with invertase. The curves, both of saccharose and reducing sugars, are far more complicated than those of the first picking (August 26th). Both curves show three well-defined synchronising maxima and minima; the maxima are at 2 p.m., 6 p.m. and 2 a.m. During the greater part of the day, viz. from sunrise to 4 p.m., the saccharose fluctuates in almost exactly

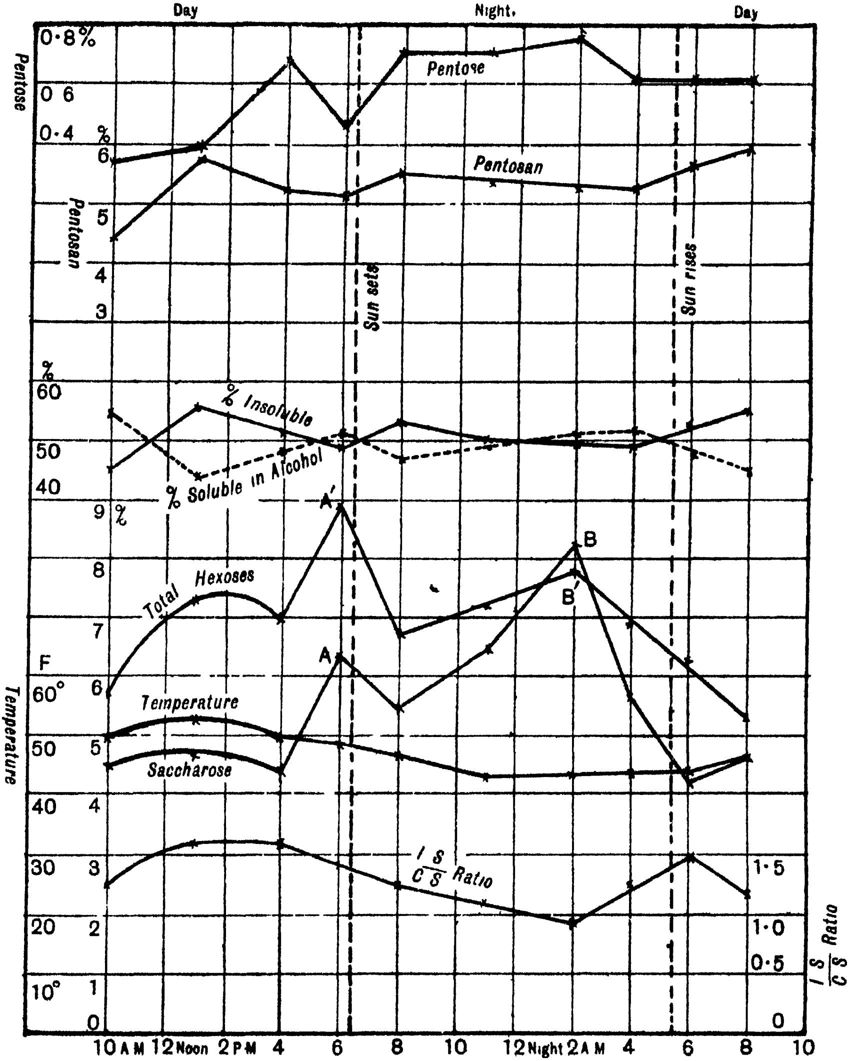

Fig. 5 Mangold leaf, Series II, Sept 10–11, 1912.

the same way as the temperature. The actual change in the amount of sugar is relatively small, probably owing to the fact that on this particular day there was a lack of direct sunshine, and a cold wind prevailed; the rise of temperature (50–53°) was slight from 10 a.m. to 1 p.m., when the maximum was reached, and the corresponding increase in saccharose was very small, viz. from 4·51 to 4·62 per cent. On the other hand the increase of hexoses takes place far more rapidly, as was the case in August, but with this exception, the general *shape* of the hexose curve is the same as that of the saccharose curve. In both cases, two peculiar maxima appear (at 6 p.m. and 2 a.m.) which were not found at the earlier stage of growth, but reappear, as we shall see later (see Fig. 6), in October. Both the cane sugar and reducing sugar after reaching a maximum at about 2 p.m., corresponding with the temperature maximum, begin to fall off slightly up to 4 p.m., when a sudden rise in both sugars occurs, just *before* sunset, maxima A and A' being reached which are considerably higher (about 50 per cent.) than the highest values previously reached. Similar maxima are found in the October picking (Fig. 6) just *after* sunset. These sudden increases in the sugars at this time of day find a parallel in the potato leaf (see p. 366, and Fig. 1) in an equally sudden increase in the starch (from 2 to 6 per cent.). It may be that the rise in the sugars may be accounted for by a cessation in their translocation from the leaf, leading to an accumulation in the leaf tissue. But from 6 p.m. to 8 p.m. both sugars are again falling; at 8 p.m. a second rise sets in, which is more rapid in the case of the saccharose than of the hexoses, the maximum B for cane sugar being actually higher than that for the reducing sugars, B'. At this point the percentages of saccharose and hexoses are nearly the same, viz., about 8 per cent.; the percentage of cane sugar at this point is far higher than at any previous hour of the day, being nearly double that corresponding with the maximum reached at 2 p.m. when the leaves were exposed to direct light, and 1⅓ times the value reached at 6 p.m. Just as the night rise of saccharose was more rapid than that of the hexoses, the falling off of the cane sugar is also more abrupt. It is noteworthy that the increase of hexoses from 8 p.m. to 2 a.m. and the subsequent decrease from 2 a.m. to 8 a.m. take place along exactly straight lines; with the cane sugar this is not strictly the case. The fall of hexose continues also some little time after sunrise, but the saccharose apparently responds at once to the daylight and increases in amount.

It is a very striking fact that although the curves for saccharose and the hexoses are of so complicated a character, the curve showing the variation of the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ (that is invert sugar to cane sugar) is relatively simple. As in the earlier stage of growth (see Fig. 4) the curve more or less closely follows the temperature curve during the day and the greater part of the night. As the temperature rises from 10 a.m. to 1 p.m., the invert sugar increases faster than the saccharose, but when the temperature falls, the ratio of invert sugar to cane sugar falls off along *practically a straight line*, exactly as was the case in Series I (Fig. 4). From 2 a.m. to 6 a.m., the saccharose is disappearing faster than the hexoses, so that the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ rises, again along nearly a straight line, until just after sunrise, when the formation of cane sugar begins more rapidly than that of hexose sugar.

It is very difficult to explain the night maxima, *B* and *B′*, which form such a striking feature of the sugar curves at this stage of growth and also in the later and final period in October (see Fig. 6, p. 289). The maxima reached at night are in the case of both sugars considerably higher than those attained during actual insolation. Both sugars increase together and fall together, so that interconversion cannot explain the result. The *sum* of the sugars at the night maximum (16·6 per cent.) is slightly higher than at 6 p.m. (15·3 per cent.) and far higher than at 1 p.m. (12·1 per cent.), when the direct formation of the sugars under the influence of light reaches a maximum. It is improbable that, at night, a reverse current of sugar sets in from the roots to the leaves and our actual analyses as well as a careful microscopic examination of all the samples have shown the entire absence of starch from the leaf during the day and night. Had starch been present, the increase in the amount of the sugars at night might be due to the transformation of starch into these. In the absence of starch, any explanation of the large increase in the proportion of soluble sugars which is observed to attain a maximum in the neighbourhood of 2 a.m. (at about 3 a.m. in October, in both cases about 3 hours before sunrise) must be more or less conjectural. Both in September (Series II) and October (Series III) the maximum concentration of the sugars is reached at nearly the same time, whilst the proportion of cane sugar is practically identical (about 8 per cent.) in both cases, in spite of the day values for saccharose being far lower in Series II than in Series III. As starch and the product of its hydrolysis. maltose, are entirely absent from the mangold leaf it seems probable that some other substance acts as a reserve at this period of growth, and, during the night, is broken down to cane sugar and invert sugar, thus causing the rise which is observed. The mangold leaf undoubtedly contains a large amount of *gummy* substance which is soluble in water and, after the attempted conversion of the starch with taka-diastase, is precipitated as a semi-crystalline mass by basic lead acetate. Whether this substance can give rise directly to cane sugar or reducing sugars on hydrolysis can only be decided by a special investigation, but it would appear that this or some kindred substance is the source of the great increase of sugars which occurs between midnight and 3 a.m., both in the September and October pickings.

As compared with the earlier picking in August the following are the outstanding features:

1. The proportions and range of variation of the sugars are considerably greater:

On August 26th–27th, the saccharose varied from 3·05 to 1·5 per cent., hexoses from 2·15 to 0·2 per cent.

On September 10th–11th the saccharose varied from 8·27 to 4·24 per cent., hexoses from 8·9 to 5·4 per cent.

This, too, in spite of the fact that September 10th was a dull, cool day unfavourable to photo-synthesis.

2. The relative position of the saccharose and hexose curves has changed; whereas in August the cane sugar curve was always *above* the hexose curve, the ratio $\frac{\text{I.S.}}{\text{C S.}}$ varying between the limits 0·13 and 0·71, in no case reaching 1·0, on September 10th–11th the hexose curve is throughout the 24 hours above the cane sugar curve, except for a moment at 2 a.m., when the two sugars are present in nearly equal amounts. During the 24 hours the $\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·94 to 1·60.

3. During the later period of the night, when the proportion of sugars is falling, the leaves became nothing like so depleted of sugars as in the earlier stage of growth; whereas on August 26th–27th the cane sugar fell to 1·5 per cent. and the hexoses practically disappeared, on September 10th–11th the lowest value reached by the cane sugar was 4·24 per cent. and by the hexoses 5·4 per cent.

Pentosans, Matter Insoluble in Alcohol and Pentoses.

As at the August picking, but in a far more unmistakable manner, the curve showing the proportion of leaf substance which is insoluble in alcohol runs *exactly parallel to the pentosan curve* (see Fig. 5). It is a striking fact that from 10 a.m. to 1 p.m., in spite of the large increase in the sugars, that is of matter soluble in alcohol, there is a large actual increase of substances which are insoluble in alcohol, and exactly parallel with it, a rise in the pentosans. It is clear therefore that the increase of pentosan material, which probably includes the gum-like substances mentioned above (p. 285), must take place at a relatively greater rate than the increase of sugars, since their increase does not mask it. Saccharose, hexoses, pentoses and pentosans are all increasing simultaneously during the first period of the day, that is whilst the temperature is rising; it is probable, as stated on p. 281, that the hexoses are converted into pentoses and the latter into pentosans. Thus we find the pentoses rising not so quickly as the pentosans whilst the latter are being formed, but from 2 p.m.–4 p.m. when the hexoses are *falling* a rapid rise of pentoses occurs, whilst the pentosans from this point up to 6 p.m. have ceased to be formed. It is probable that the sudden apparent *fall* followed by a *rise* of pentoses between 4 p.m. and 8 p.m. is partly a relative effect owing to the large sudden increase and decrease of the saccharose and hexoses between these points; but this would not account for the magnitude of the change (from 0·68 to 0·45 per cent. and back again to 0·71 per cent.), and the two changes are obviously interconnected and take place in opposite directions. From 8 p.m. to 2 a.m. there is an actual rise of pentoses, which must be somewhat greater than it appears because it is partly masked by the large increase of saccharose and hexoses during this interval. The fall of pentosans between 8 p.m. and 4 a.m. is probably only an apparent or relative effect, owing to the large increase in the other sugars, but the rapid rise of pentosans from 4 a.m. onwards corresponds with the actual fall of pentoses from 2 a.m. onwards, which must be larger than it appears because the saccharose and hexoses are falling simultaneously.

The actual range of pentoses during the day is small, viz. 0·34–0·76 per cent.

Series III. Final Stage of Growth, October 11th–12th, 1912.

Changes during the 24 *hours.*

Maltose and *Starch* are again entirely absent.

Saccharose and Hexoses. These increase immediately after sunrise, the curves (see Fig. 6) rising at first almost parallel to the temperature curve; the cane sugar increases in amount until midday but from this point until sunset (5 p.m.) the *total* quantity of sugars present remains nearly constant, the fluctuations between 11 a.m. and 5 p.m. consisting merely of interconversions of the cane sugar and invert sugar. Thus between 1 p.m. and 3 p.m. cane sugar increases, apparently at the expense of the reducing sugars, whilst from 3 to 5 p.m. cane sugar falls and the hexoses increase in amount. At sunset, as in the August picking, a rapid rise of both cane sugar and reducing sugars occurs, the cane sugar reaching its maximum first at 7 p.m. and then apparently being converted into hexoses; this is shown by the rise of hexoses between 7 and 9 p.m., whilst the saccharose is falling. From 9 p.m. to 11 p.m. both sugars are falling, but between 11 p.m. and 3 a.m. there is again a rapid rise in the sugars, exactly as in the September picking, until the night maxima, *B* and *B′*, are reached, at 3 a.m., that is 3 hours before sunrise, a position

TABLE III. *Mangold Leaves*, October 11th–12th, 1912.

Oct. 11th. Sun rises 6.19 a.m. Sun sets 5.15 p.m. Oct. 12th. Sun rises 6.21 a.m. Sun sets 5.13 p.m.

Time	Temp.	% of leaf*		Sacc. % in T.V.D.M.		Δ = C.A. − I.	Saccharose av. %	Hexoses %† as I.S.	Saccharose + hexoses	Pentose %	Pentosan %	Maltose	Starch	I.S./C.S. (av.)	Remarks
		Soluble in alcohol	Insoluble in alcohol	Citric acid	Invertase										
9 a.m.	40° F.	52·2	47·8	5 67	5·38	+0·29	5·53	10·32	15·85	0·82	6·89	0·00	0·00	1·87	Foggy night, sun getting through at 9 a.m.
11 a m.	55°	54·6	45·4	7·29	7·02	+0·27	7·16	11·62	18·78	0·91	6·21	"	"	1·62	Warm and sunny
1 p.m.	61°	52·5	47·5	7·19	6·83	+0·36	7·01	12·12	19·13	0·92	6·59	"	"	1·73	Very sunny
3 p.m.	58°	54·9	45·1	8·92	9·11	−0·19	9·02	10·24	19·26	0·86	6·35	"	"	1·14	Sunny
5 p.m.	50°	52·5	47·5	7·64	7·41	+0·23	7·52	11·46	18·98	0·86	6·68	"	"	1·52	Cooler, hazo
Dark 6.30															
7 p.m.	44°	54·0	46·0	9·48	9·56	−0·08	9·52	11·47	20·99	0·92	6·60	"	"	1·20	Hazy
9 p.m.	42°	54·2	45 8	7·41	7·16	+0·25	7·28	11·98	19·26	0·84	6·65	"	"	1·65	Hazy
11 p m	39°	47·9	52·1	6·80	6·78	+0·02	6·79	9·39	16·18	0·68	7·15	"	"	1·38	Slight fog
1 a m	38°	52·6	47·4	7·15	6·68	+0·47	6·92	10·78	17·70	0·80	7·09	"	"	1·56	Cold and foggy
3 a m	36°	55·9	44·1	8·40	8·42	−0·02	8·41	12·41	20·82	0·70	6·78	"	"	1·48	Ice on leaves
5 a m.	31°	54·7	45·3	6·93	6·88	+0·05	6·91	11·49	18·40	0·70	6·77	"	"	1·67	Ice thick on leaves
1st light 5.30															
7 a.m.	35°	51·6	48·4	5·08	4·88	+0·20	4·98	9·62	14·50	0·61	6·77	"	"	1·93	Leaves frozen stiff

* Calculated on the total vacuum-dried weight of leaf.

† *Allowance has been made for the pentoses present.*

which agrees very closely with that found in Series II. After these maxima have been reached (probably as in the August picking, owing to certain reserve substances, such as gums, being put under contribution), the sugars continue to fall at almost parallel rates and along practically straight lines until just after sunrise.

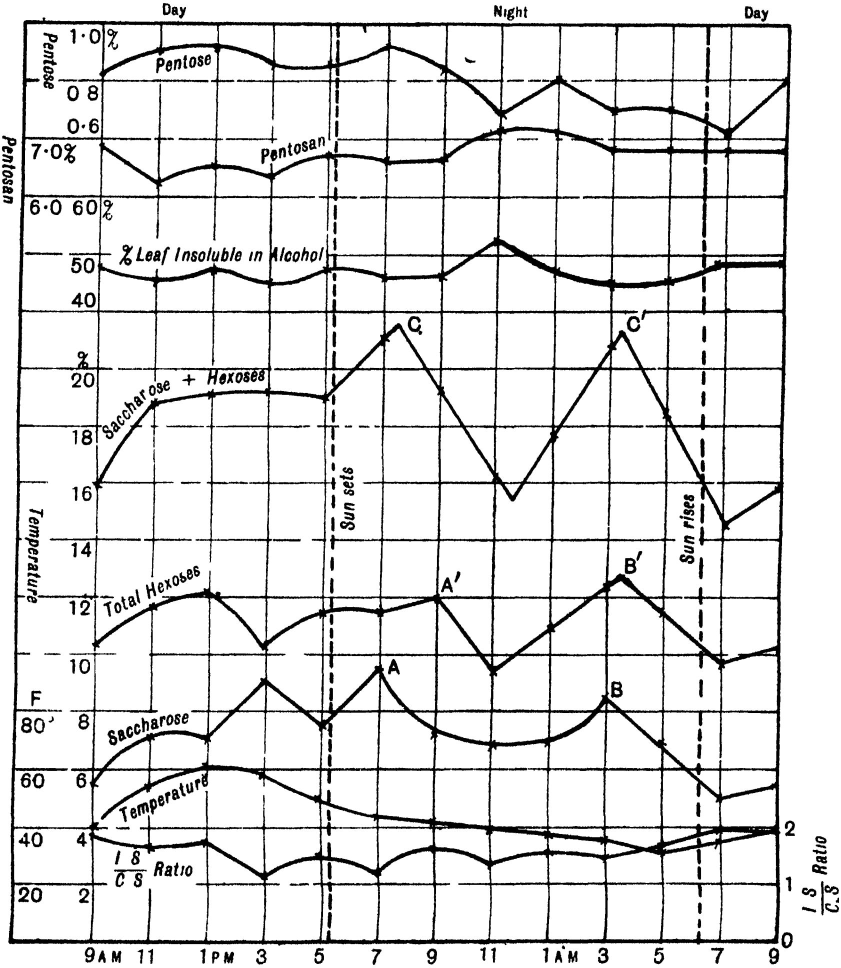

Fig. 6. Mangold leaves, Series III, Oct. 11–12, 1912.

It is a striking fact that the two maxima for the *total* sugars (*C* and *C′*) at night correspond with roughly the same proportion of sugars (about 21 per cent. of the total vacuum-dried matter); the same was true of the two night maxima of Series II, but here the value for the total sugars was much lower, viz. about 16 per cent. It is also striking that the successive rises and falls in the proportion of *total* sugars at night occur along practically straight lines (a phenomenon also observed in Series II, although the curve of total sugars is not shown in Fig. 5).

Fluctuation of the $\frac{I.S.}{C.S.}$ *ratio.*

In the final stage of growth in October, although the general type of the sugar curves is quite similar to that of Series II, the curve showing the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ is of an entirely different character. Whereas in Series I and II, during the greater part of the day, the curve showing the ratio of the two sugars runs very nearly parallel to the temperature curve, in the last stage of growth the curve shows a periodic increase and decrease of the ratio which the two sugars bear to one another. From 9 a.m. to 11 a.m. saccharose increases faster than hexoses. from 11 a.m. to 1 p.m. the reverse is true; then comes a rapid increase of saccharose and a fall of hexoses until 3 p.m., which is reversed between 3 and 5 p.m. At night there is a similar periodic fluctuation, and, exactly as in Series II, when the cane sugar increases at night it does so far more rapidly than the hexoses, cane sugar apparently being the primary product which is formed from the reserve substance, and not hexoses. On the other hand, exactly as in September, the saccharose falls off, from 3 a.m. to sunrise, considerably more rapidly than the invert sugar, so that the curve $\frac{\text{I.S.}}{\text{C.S.}}$ rises steadily; as in Series II, the rise is almost along a straight line. This is perhaps due to the inversion of the cane sugar being effected by invertase more rapidly than the invert sugar formed is consumed by the changes to which it is subjected. It is noteworthy that these changes, in this case, take place when the external temperature is below freezing-point; at 3 a.m. ice was forming on the leaves and at 7 a.m. they were all frozen stiff.

Pentosans, leaf-matter insoluble in Alcohol and Pentoses. As in Series I and II, the curve of matter insoluble in alcohol is parallel to the pentosan curve. At this stage of growth, however, the variation during the day is much less marked than in September (Series II), the insoluble matter ranging from 45–48 per cent., the pentosan from 6·2 to 6·9 per cent.; the greater part of the variation seems to be purely relative, that is the apparent fall of insoluble matter between 9 a.m. and 11 a.m. corresponds with the large increase of sugars (15·8 to 18·8 per cent.) between these hours. This is very different from the August and September pickings when a rapid formation of the pentosans (gums or ligneous tissue) was clearly obvious in spite of the simultaneous increase in the sugars. A certain amount of pentosan formation does apparently occur, however, even at this later stage of growth, and is visible in the rise between 11 a.m. and 1 p.m. The fluctuations at night seem to be principally relative—thus the increase in the matter insoluble in alcohol which occurs between 7 p.m. and 11 p.m. is due to the rapid fall of sugars from 21 to 16 per cent., but as there is a fall of the pentose curve between the same points, it appears that some real pentosan formation occurs from these sugars. The fall of pentosan and insoluble matter from 11 p.m. to 3 a.m. is also a relative effect due to the increase of the sugars and the rise from 3 a.m. to 7 a.m. is also relative, owing to the falling off of these.

The free *pentoses* increase during the day as in the earlier pickings and follow very largely the invert sugar curve; thus there is a continuous increase from 7 a.m. to 1 p.m., after which the pentose falls, apparently giving rise to pentosan. At night the pentose falls between 7 and 11 p.m. and the pentosan rises; the sudden rise of pentose between 11 p.m. and 1 a.m. occurs simultaneously with the sudden increase of hexoses. The fall of pentoses subsequently to this, from 1 a.m. to 3 a.m., is partly a relative effect, due to the rise in the reducing sugars.

The total variation of the pentoses during the day is only very small, viz. 0·82 to 0·92 per cent., but at night the fluctuations (probably largely relative) are from 0·92 to 0·61. During the day the fluctuations are considerably less than during the daytime in Series II; but at night the changes are greater, mainly representing a falling off in the pentoses.

Comparison of Series III with Series II.

1. The actual proportions of saccharose and hexoses and the range of variation of these sugars are considerably greater in Series III (October 11th–12th) than in Series II (September 10th–11th) and therefore far greater than in Series I (August 26th–27th).

September 10th–11th. Cane sugar varied from 8·27 to 4·24 per cent.; hexoses, 8·9 to 5·4 per cent.

October 11th–12th. Cane sugar varied from 9·52 to 4·98 per cent.; hexoses, 12·41 to 9·39 per cent.

In both series the cane sugar varies between wider limits than the hexoses, and the difference is most marked in Series III.

2. As in the September picking the curve of hexoses is always well above the curve of saccharose, the hexose being always largely in excess of the cane sugar. Consequently, the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ is always greater than unity; it fluctuates between the values 1·14 and 1·93; in September it ranged between 0·94 and 1·60, whilst in August the range was 0·13 to 0·71.

3. Series III differs from Series II mainly in the fact that during the earlier part of the day (9 to 11 a.m.) the saccharose increases faster than the hexoses, as shown by the falling $\frac{\text{I.S.}}{\text{C.S.}}$ ratio; subsequently the total sugars remain nearly constant in amount until after sunset. The periodic fluctuation of the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ and the mutual interconversion of cane and invert sugar which it expresses are characteristic of the final stage of growth only. Whereas, too, in the earlier pickings the proportion of the *total sugars* falls off rapidly after the temperature maximum has been reached until the rise occurs in the neighbourhood of sunset, in this case the total sugars remains nearly constant during the whole afternoon, a balance being reached such that the sugars removed from the leaf are equal in quantity to those being formed in it. This characteristic of the last stages of growth is apparently due to the root having relatively less capacity to remove the sugars from the leaf than at earlier stages, in August and September the root was capable of removing the sugars from the leaf in the afternoon faster than they were formed, in October, the out-take and production just balance one another.

4. The total sugars present are at a far higher level in Series III than in Series II; in Series II they formed 10 to 16 per cent., and in Series III from 15 to 21 per cent. of the total vacuum-dried leaf matter.

Variations during Complete Period of Growth.

In Table IV we give the diurnal variations of the leaf carbohydrates for the three stages of growth investigated.

The principal conclusions which can be drawn as to the total variations during growth are as follows:

1. The proportions of all the sugars present in the leaves increase progressively from the first to the final stage of growth; this is true of saccharose, hexoses and pentoses. The extreme diurnal variation is, however, greatest for the hexoses, pentoses, pentosan and matter

insoluble in alcohol, at the September picking (Series II); it would probably have been the same also for the saccharose had not the day been abnormally dull and cloudy, so that the range of temperature was exceedingly small ($\Delta = 7°$) and the increase of cane sugar (which follows the temperature curve) correspondingly small (see Fig. 5).

TABLE IV. *Range of variations during growth.*

Series and date	Temp. °F.	Saccharose %	Hexoses as invert sugar %	Pentose %	Pentosan %	Ratio $\frac{\text{I.S.}}{\text{C.S.}}$	Matter insoluble in alcohol %	Saccharose + hexoses %
I. Aug. 26–27	45–75 Δ=30	1·50–3·11 Δ=1·61	0·20–2·16 Δ=1·96	0·36–0·52 Δ=0·16	5·19–5·96 Δ=0·77	0·13–0·71 Δ=0·58	57·5–62·9 Δ=5·4	1·70–5·26 Δ=3·56
II. Sept. 10–11	43–50 Δ=7	4·24–8·27 Δ=4·03	5·38–8·90 Δ=3·52	0·34–0·76 Δ=0·42	4·42–5·90 Δ=1·48	0·94–1·60 Δ=0·66	45·3–55·8 Δ=12·5	9·98–16·08 Δ=6·1
III. Oct. 11–12	31–61 Δ=30	4·98–9·52 Δ=4·54	9·39–12·41 Δ=3·02	0·61–0·92 Δ=0·31	6·21–7·15 Δ=0·94	1·14–1·93 Δ=0·79	45·1–52·1 Δ=7·0	14·5–20·99 Δ=6·49

2. The proportion of *pentosan* appears to fall slightly from the first to the second Series, but then increases from the second to the third. The apparent fall is really a relative effect, due to the large increase in sugars and other soluble substances which are formed between the dates of the first and second pickings. If the pentosans are calculated as percentages of the vacuum-dried leaf matter which is insoluble in alcohol, we get a steady increase in the amount of pentosan constituent as the season advances, as follows:

Series I. Pentosans form 8·58–9·61 per cent. of the insoluble leaf matter.

Series II. Pentosans form 9·83–10·85 per cent. of the insoluble leaf matter.

Series III. Pentosans form 13·70-15·35 per cent. of the insoluble leaf matter.

In passing from Series II to Series III, a very large increase in the proportion of pentosan constituents occurs, pointing probably to an increase in lignification during the interval.

3. The *hexoses* more and more predominate in the leaf as the season advances; at first they form only a fraction of the saccharose ($\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·13 to 0·71 in Series I), but later on they become equal

to and even nearly double the saccharose $\left(\frac{\text{i.s.}}{\text{c.s.}} = 0{\cdot}94\text{–}1{\cdot}60 \text{ in September, and } 1{\cdot}14 \text{ to } 1{\cdot}93 \text{ in October}\right)$.

An exactly similar increase in the proportion of reducing sugars was observed by Parkin [1912] in the case of the snowdrop (*Galanthus nivalis*).

4. Whereas in the first stage of growth practically *all* the reducing sugar and about one-half of the cane sugar are used up in the night, in the later stages of growth only a small part of these sugars disappears in the night, so that each day's activity commences with a larger proportion of total sugars. This is well seen from the following data:

	% hexoses	% saccharose
At sunrise, August 27th	0·20	1·50
„ September 11th	6·30	4·24
„ October 12th	9·62	4·98

The store of reducing sugars which is thus available at the commencement of the day steadily and rapidly grows, especially in the earlier part of the season: but the store of cane sugar in the leaf, although increasing rapidly from August 27th to September 11th seems to reach a nearly stationary value. Table IV shows that the *limits* between which the sugars vary constantly rise during the season, but not so much in the case of the cane sugar as in that of the reducing sugars; but the range of variation *for the* 24 *hours* increases considerably more in the case of the saccharose than in that of the hexoses. It must be noted, however, that the range of variation during the daylight period, up to the time of reaching the first maximum (which corresponds with the temperature maximum), is always greater in the case of the hexoses than the saccharose, and especially so in the first two series; when growth of the root is nearly complete, the range of variation of the cane sugar in the leaf during the period of illumination becomes far greater. In this case, when the root has nearly reached the limit of its storing capacity, the leaf itself seems to act as a temporary reservoir of cane sugar. This is shown by the following data:

Daylight Variations.

	Saccharose	Hexoses
Series I	2·51–3·11 Δ = **0·6** %	0·20–2·16 Δ = **1·96** %
Series II	4·24–4·62 Δ = **0·38** %	5·38–7·50 Δ = **2·12** %
Series III	4·98–7·16 Δ = **2·18** %	9·62–12·12 Δ = **2·50** %

B. THE SUGARS OF MID-RIBS AND STALKS (PETIOLES). THE TRANSLOCATION OF THE SUGARS.

Series I. August 26th–27th, 1913.

In this series, the leaf-stalks (petioles) were divided into top and bottom halves, and the two sets were treated separately; in this way it was hoped that any change in the saccharose and hexoses during their passage to the root might be detected. Actually it was found that very considerable differences exist in the composition of the sap in the two halves, as shown by the data in Table V.

TABLE V. *Series I.* August 26th–27th, 1913.

Top and Bottom Halves of Mangold Leaf-stalks.

Time		% of stalk soluble in alcohol	% saccharose on T.V.D.M. by		Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf		$\frac{\text{I.S.}}{\text{C.S.}}$		Pentose %	Pentosan %	Maltose %	$R = \frac{\text{I.S.} + \text{C.S.}}{\text{Matter sol. in alc.}}$ %
			Citric acid	Invertase					Saccharose	Hexoses	In stalk	Leaf				
6 a.m.	Tops	52·9	3·36	4·14	−0·78	3·75	5·35	9·10	2·51	0·77	1·42	0·31	1·32	10·56	0·00	17·2
	Bottoms	54·3	3·47	3·89	−0·42	3·68	9·11	12·79	,,	,,	2·48	,,	1·20	10·16	,,	23·5
12 noon.	Tops	54·9	4·52	4·26	+0·26	4·39	9·97	14·36	3·11	2·15	2·27	0·69	0·89	10·00	0·00	26·1
	Bottoms	58·3	—	4·12	—	4·12	13·17	17·29	,,	,,	3·20	,,	1·50	9·70	,,	29·6
6 p.m.	Tops	53·6	4·14	3·92	+0·22	4·03	7·89	11·92	2·82	0·96	1·95	0·34	1·20	10·20	0·00	22·2
	Bottoms	57·8	4·03	4·09	−0·06	4·06	10·47	14·51	,,	,,	2·58	,,	1·21	9·65	,,	25·1
12 night.	Tops	50·6	4·12	3·98	+0·14	4·05	6·61	10·66	2·18	0·57	1·63	0·26	1·13	10·47	0·00	21·1
	Bottoms	55·8	—	4·15	—	4·15	8·49	12·64	,,	,,	2·04	,,	1·06	9·82	,,	22·6

In Series II the mid-ribs of the leaves were cut out and dealt with separately. The stalks in this series were treated as a whole, and not subdivided into top and bottom halves.

A careful comparison of the data in Tables V, VI, VII and VIII, and Figs. 7 to 10 gives important information as to the translocation of the sugars from the leaf to the root. The following are the principal points disclosed:

1. The proportion of sugars and of matter soluble in alcohol is always far greater in the stalks and mid-ribs than in the leaves at the same picking: thus, for example, in Series I, 12 noon,

Bottoms of stalks contained 17·29 per cent. total sugars,
58·3 per cent. matter soluble in alcohol;
when the *Leaf* contained 5·26 per cent. total sugars,
40·6 per cent. matter soluble in alcohol.

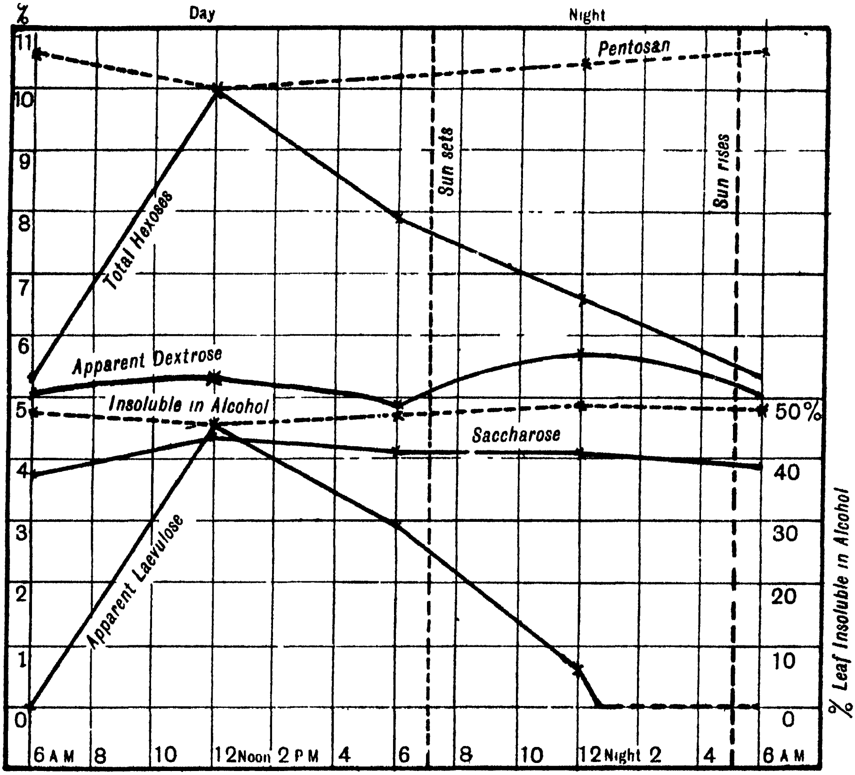

Fig. 7. Mangold stalks, tops, Series I. Aug. 26–27, 1913.

2. As the season advances, a great increase occurs in the proportion of sugars in the stalks at corresponding times of day. Thus, for example, we have:

At 12 noon, August 26th. Total sugars = 15·8 per cent.; matter soluble in alcohol = 56·6 per cent. (average of tops and bottoms).

At 10 a.m., September 10th. Total sugars = 25·3 per cent.; matter soluble in alcohol = 65·4 per cent.

At 11 a.m., October 11th. Total sugars = 30·99 per cent.; matter soluble in alcohol = 63·0 per cent.

In this comparison, in the last two cases samples were not taken at noon, but had they been, the differences would be even greater.

TABLE VI. *Series II.* September 10th–11th, 1912.

Stalks.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by		Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf		$\frac{\text{I.S.}}{\text{C.S.}}$		Pentose %	Pentosan %	Maltose %	$R = \frac{\text{I.S.} + \text{C.S.}}{\text{Matter sol. in alc.}}\%$
		Citric acid	Invertase					Saccharose	Hexoses	In stalk	In leaf				
10 a.m.	65·4	5·25	4·39	+0·86	4·82	20·5	25·32	4·51	5·72	4·25	1·27	1·11	8·01	0·00	38·7
4 p.m.	66·9	5·75	4·78	+0·97	5·26	26·3	31·56	4·38	7·00	5·00	1·60	1·16	7·98	,,	47·2
11 p.m.	65·4	5·18	—	—	5·18	22·4	27·58	6·41	7·10	4·33	1·11	1·06	8·65	,,	42·3
4 a.m.	64·2	5·34	5·10	+0·24	5·22	23·75	28·97	5·62	6·91	4·55	1·23	1·02	8·43	,,	45·1
6 a.m.	64·4	5·25	4·88	+0·37	5·06	26·7	31·76	4·24	6·30	5·27	1·48	1·10	8·84	,,	49·3

TABLE VII. *Series II.* September 10th–11th, 1912.

Mid-ribs.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by		Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf		$\frac{\text{I.S.}}{\text{C.S.}}$		Pentose %	Pentosan %	Maltose %	$R = \frac{\text{I.S.} + \text{C.S.}}{\text{Matter sol. in alc.}}\%$
		Citric acid	Invertase					Saccharose	Hexoses	In stalk	In leaf				
10 a.m.	60·9	6·47	6·22	+0·25	6·35	23·6	29·95	4·51	5·72	3·72	1·27	0·50	10·28	0·00	49·2
4 p.m.	62·8	6·53	5·62	+0·91	6·08	22·6	28·68	4·38	7·00	3·72	1·60	0·98	9·84	,,	45·7
11 p.m.	58·3	6·79	6·58	+0·21	6·68	20·6	27·28	6·41	7·10	3·08	1·11	1·11	10·92	,,	46·8
4 a.m.	64·8	7·42	7·46	−0·04	7·44	19·0	26·44	5·62	6·91	2·55	1·23	1·25	9·52	,,	40·8
6 a.m.	60·3	6·50	6·40	+0·10	6·45	21·4	27·85	4·24	6·30	3·32	1·48	1·07	10·66	,,	46·2

TABLE VIII. *Series III.* October 11th–12th, 1912.

Stalks and Mid-ribs.

Time	% of stalk soluble in alcohol	% saccharose on T.V.D.M. by		Δ = C.A. − I.	Av. value saccharose %	Hexoses %	Saccharose + hexoses %	In leaf		$\frac{\text{I.S.}}{\text{C.S.}}$		Pentose %	Pentosan %	Maltose %	$R = \frac{\text{I.S.} + \text{C.S.}}{\text{Matter sol. in alc.}}\%$
		Citric acid	Invertase					Saccharose	Hexoses	In stalk	In leaf				
Stalks:															
11 a.m.	63·0	5·58	5·00	+0·58	5·29	25·7	30·99	7·16	11·62	4·86	1·62	1·01	9·28	0·00	49·2
11 p.m.	61·1	5·54	5·19	+0·35	5·36	21·4	26·76	6·79	9·39	3·99	1·38	1·28	9·51	,,	43·8
Mid-ribs:															
11 a.m.	63·95	6·94	—	—	6·94	22·5	29·44	7·16	11·62	3·24	1·62	1·0	9·66	0·00	46·0
11 p.m.	59·5	6·72	—	—	6·72	18·5	25·22	6·79	9·39	2·75	1·38	1·0	10·84		49·4

3. The values of *R* in the last column, giving the percentage of total sugars in the matter extracted by alcohol, show that as the season advances the sugars form a larger and larger proportion of the total soluble matter which is conveyed by the stalks and mid-ribs. In Series I, August 26th, the values of *R* range from 17·2 to 29·6 per cent.; whereas in September and October the sugars formed 40 to 50 per cent. of the total matter soluble in alcohol. The proportion of sugars in this dry matter is at a minimum early in the morning (6 a.m.) and at a

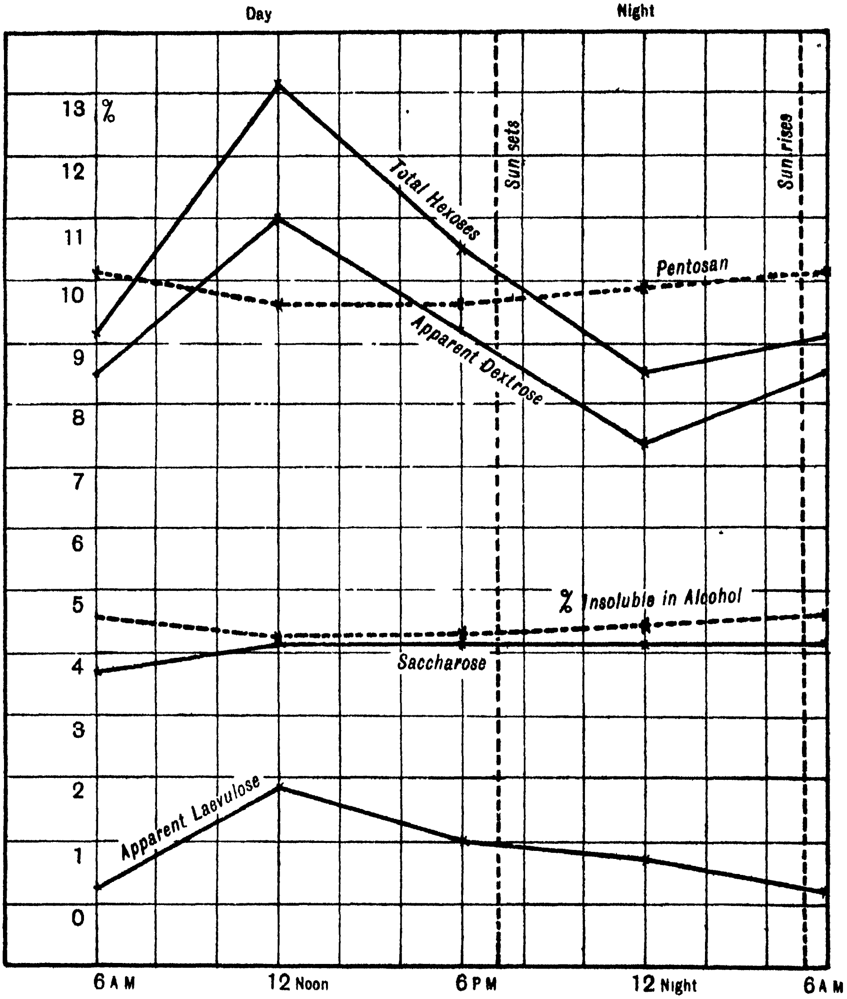

Fig. 8. Mangold stalks, bottoms, Series I, Aug. 26–27, 1913.

maximum about mid-day, exactly as in the leaves; after reaching the maximum, the sugars (that is the *hexoses*, the saccharose *being practically constant all day*) fall off steadily, almost along a straight line. In the *top* half of the stalks this falling off in the proportion of sugars continues all the night through, but in the bottoms it continues only till midnight, when a slight rise in the proportion of the sugars occurs, owing probably to the rate of inflow from above being greater than the outflow into the roots (see Figs. 7 and 8)

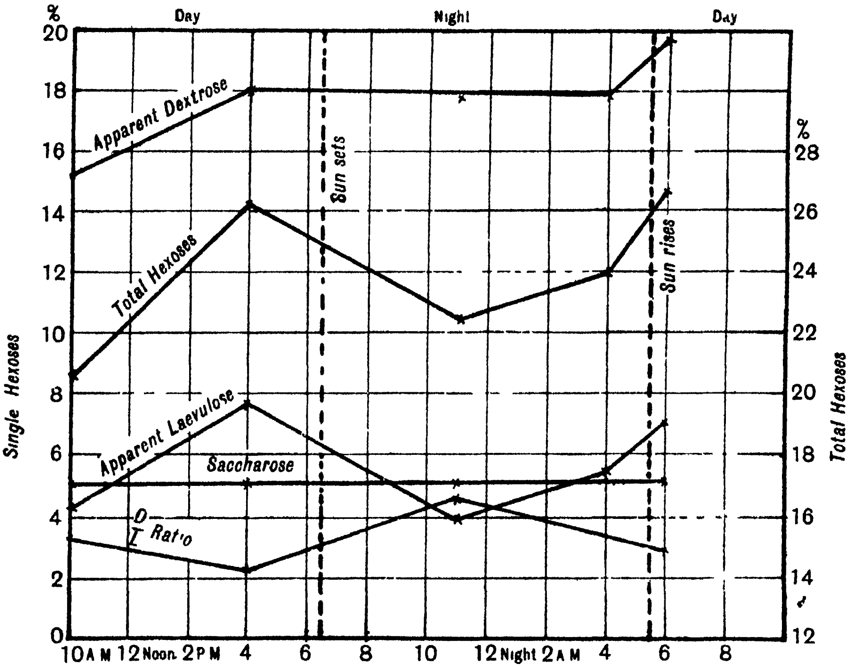

Fig 9 Sugars in stalks, Mangolds, Series II. Sept 10–11, 1912.

In the September picking (Series II) (Fig. 9) the saccharose is again practically constant throughout the 24 hours, but the total hexose increases from 10 a.m. to 4 p.m., corresponding with the increase of the leaf sugars which occurs during this interval; at 4 p.m. a falling off of the hexoses occurs in the stalks, which lasts until nearly 11 p.m., and this coincides with the increase of the cane sugar and hexoses in the leaf which occurs at 4 p.m. (see Fig. 5), and was assumed to be due probably to a cessation of translocation from the leaf. It is interesting to note that the large increase in both saccharose and hexoses, which occurs in the leaf between sunset and 2 a.m. (Fig. 5) and was assumed to be due to the transformation of reserve substances (gums) into sugars, finds its counterpart in the stalk curve (Fig. 9) in the *increase* of the hexoses which starts at night at about 11 p.m. and continues until sunrise; this increase of the sugars in the stalks, occurring moderately rapidly between 11 p.m. and 4 a.m., and then far more rapidly from 4 a.m. to 6 a.m., exactly corresponds with the *rising* portion of the sugar curves in Fig. 5 from 8 p.m. to 2 a.m., followed by the rapid fall from

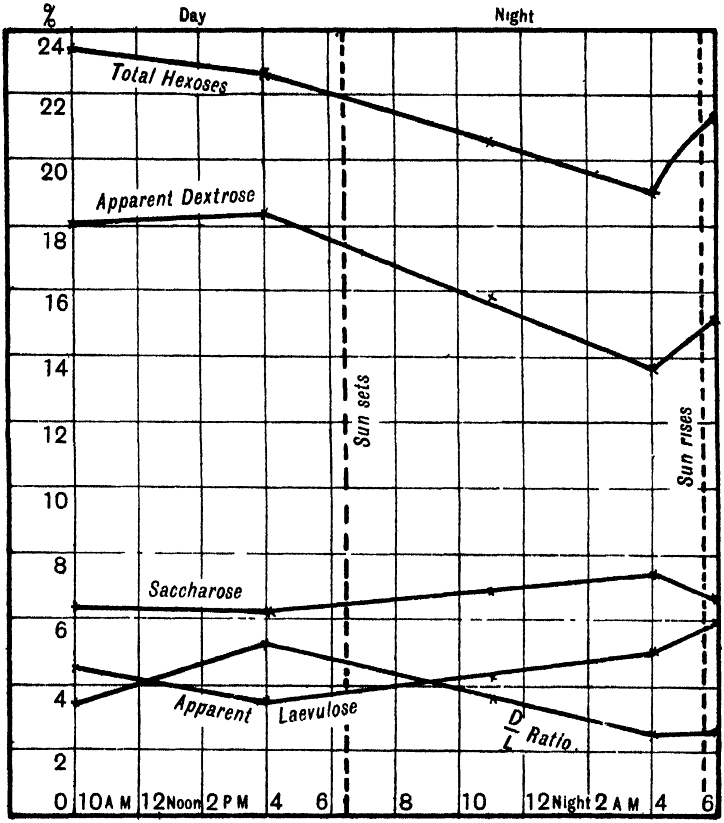

Fig. 10. Mangold mid-ribs, Series II, Sept. 10–11, 1912.

the maxima *B* and *B′*, which occurs between 2 a.m. and 6 a.m. The rapid fall of the sugars in the leaf is no doubt largely due to their translocation from the leaf to the top of the stalks. It is important to observe (comparing Figs. 9 and 10) that, as might be anticipated if the sugars are formed in the leaf tissue and are thence conveyed to the mid-ribs and stalks, the movement of the sugars in the mid-ribs is always in advance of the movement in the stalks; thus in Series II, when the sugars are rapidly increasing in the leaf in the morning, the total sugars in the *mid-rib* have a higher value (29·95 per cent. at 10 a.m.) than in the *stalks* (25·32 per cent.), the proportion of sugars to total alcohol-soluble matter being much higher also (49·2 per cent. as compared with 38·7 per cent.). But by 4 p.m. a large proportion of the sugars which were in the mid-rib in the morning have passed into the stalk, so that the numbers *are now reversed* (28·68 per cent. in mid-ribs, 31·56 per cent. in stalks for the sugars; 45·7 and 47·2 per cent. for the proportion of sugars to total alcohol-soluble substances). At 11 p.m. stalks and mid-ribs are practically identical; at 4 a.m. the large accumulation of sugars in the leaf at 2 a.m. is already passing out of the mid-rib and is accumulating in the stalk, thus increasing the proportion of sugars therein, this being shown by the three successive values for total sugars in the stalks, 27·58, 28·97 and 31·76 at 11 p.m., 4 a.m. and 6 a.m. By 10 a.m. a large proportion of the sugar conveyed to the stalk has passed on to the *root*, so that the proportion of sugars falls to its minimum at about this hour.

The problem of translocation is complicated by the fact that several operations are actually occurring simultaneously and the actual analytical data only give the net results of all these; thus sugars are, during the daytime, being formed in the leaves, but at the same time are passing *from* the leaves into the mid-ribs and stalks; it has been shown above that the top and bottom halves of the stalks have very different compositions and the relationship between the sugars in the mid-ribs and stalks and the top and bottom halves of these stalks is again complicated by the fact that the roots are continuously receiving the sugars from the lower part of the stalks, and the tops of the stalks from the mid-ribs. It is interesting in this connection to compare the curves for the total hexoses in the stalks in Series II with those for the mid-ribs in the same series, Figs. 9 and 10. Whereas in the daytime the hexose in the stalks first *increases*, keeping pace with the increased formation in the leaf, and then falls, in the *mid-ribs* the hexose falls continuously throughout the day and night till about 4 a.m. It thus appears that the removal of the sugars from the mid-ribs to the stalks during the day takes place somewhat faster than the sugars pass into the ribs from the leaf tissue. It is seen too that the saccharose content increases somewhat in the mid-ribs during the night, whereas in the stalks it remains practically constant. Both facts are probably due to a common cause, which comes out more clearly from later considerations, that the sugar has to pass from the mesophyll into the veins and mid-ribs in the form of the less rapidly diffusible sugar saccharose, and is there inverted to reducing sugars; these pass downwards towards the root at a greater velocity than the cane sugar can enter to take their place

4 The clearest light on the nature of the first sugar formed in the leaf and the changes which occur in translocation is obtained by comparing the values obtained for the ratio $\frac{\text{I S}}{\text{C S}}$ in the leaves, mid-ribs and top and bottom halves of the stalks at the same hour of the day Such a comparison shows at once that *the proportion of hexoses to cane sugar is always very small in the leaf as compared with its value in the mid-rib, that it is less in the mid-rib than in the stalk and less in the top half of the stalk (nearest the leaf) than in the bottom half nearer the root* Thus to take one instance only, on August 26th at 6 a m the value of the ratio $\frac{\text{I S.}}{\text{C S}}$ is only 0·307 in the leaf, whereas it is 1·42 in the tops of stalks and 2 48 in the bottoms It is unfortunate that in Series I no mid-ribs were examined, but on comparing Table VI with Table VII and the values for stalks and mid-ribs in Table VIII, it is seen that the proportion of hexoses to saccharose $\left(\frac{\text{I S}}{\text{C S}}\right)$ *is always far higher in the stalks than in the mid ribs at the same time of day* The stalks in these cases (Series II and III) were the whole stalks, so that the results give merely the average values throughout their length As seen from Table V, the proportion of hexose in the stalks rapidly increases in passing down the stalks towards the root A comparison of Table VI with Table V also shows how greatly the ratio of hexoses to saccharose increases in the stalks as the season advances and the storage of sugar in the root becomes more and more the predominating function of the plant; thus for example

At 6 a m , August 26th		At 6 a m , September 10th		
Value of $\frac{\text{I S}}{\text{C S}}$		Value of $\frac{\text{I S}}{\text{C S}}$		
In leaf	In stalks (average of tops and bottoms)	In leaf	Mid ribs	Stalks
0 307	1 95	1 48	3 32	5 27

Discussion of Results.

What is the primary sugar formed in photosynthesis in the leaf, and in what form are the sugars conveyed to the root?

The results obtained above for the increase of the ratio of hexose to saccharose in passing successively from the leaf to mid-ribs and stalks suggest unmistakably that the cane sugar is formed in the leaf and undergoes a regular and increasing amount of inversion as it passes downwards to the root. Thus at the September picking (4 p.m.), when there was $1\frac{1}{2}$ times as much hexose sugar as saccharose in the leaf, the mid-ribs contained $3\frac{3}{4}$ and the stalks 5 times as much reducing sugar as cane sugar. Thus as an average of the *whole length* of the stalks the cane sugar formed only $\frac{1}{8}$ of the total sugars; bearing in mind the results in Table V, which show how rapidly the proportion of hexose increases in passing from the top to the bottom halves of the stalks, it is clear that the sap in immediate proximity to the root contains probably only $\frac{1}{20}$ or $\frac{1}{50}$ even of its total sugars in the form of cane sugar. This fact taken in conjunction with the relatively high proportion of the cane sugar in the leaf[1] suggests the almost irresistible conclusion that the cane sugar is formed as a primary product in the leaf and is converted into invert sugar for the purpose of rapid transit; this conversion takes place apparently in the veins of the leaf, in the mid-ribs and in the stalks, as the sap travels on its way to the root, and the proportion of sugar inverted steadily increases as the sap nears the root, until in its immediate neighbourhood practically the whole of the sugar is in the form of reducing sugar. The gradual inversion of the cane sugar is probably brought about by the enzyme invertase which is secreted by or distributed on the surface of the sieve tubes, which seem to be the main channels by which the sugar is conveyed to the root (see Peklo [1908]). In accordance with this view is the fact observed by Robertson, Irvine and Dobson [1909][2] that invertase is abundant in the leaf and stem of *Beta vulgaris*, but is absent from the root.

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[1] It must be borne in mind that the leaf tissue analysed contained all the smaller veins which could not be removed by the rough method we used; only the large primary mid-ribs were removed. It is probable therefore that the hexoses found in our leaf analyses were mainly present in these veins.

[2] Colin [1915] has independently shown the presence of invertase in the leaves and petioles of the leaf of *Beta vulgaris;* he has established moreover the important fact, that the proportion of invertase is greatest in the leaves, less in the upper part of the stalks and practically nil at the base of the stalk, where it enters the root. In the root itself invertase is entirely absent, as was found to be the case by Robertson, Irvine and Dobson.

The fact that during the *early* stages of growth, when leaf formation is the principal function of the plant and the roots are merely small tap-roots, the cane sugar in the leaf is always in large excess of the hexoses (the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varying between 0·13 and 0·71) also points strongly to the cane sugar being a primary product and the hexoses as being formed by inversion from this sugar. We show in a separate paper (p. 329) that the proportions of dextrose and laevulose in the mixture of reducing sugars is always nearly that corresponding with this view. In September and October, when the call upon the cane sugar in the leaf is actually greatest for purposes of storage in the root, the ratio of hexoses to saccharose in the leaf rises; it is 0·94–1·59 in September and 1·20–1·95 in October. The relative position of the saccharose and hexose curves thus entirely changes as the function changes; in August the invert sugar curve was much below the cane sugar curve, but in September the positions were reversed. In October, the curve of hexoses was still further above the curve of cane sugar.

The facts we have brought forward as to the translocation of the sugars in the mid-ribs and stalks to our mind outweighs all the other arguments which have hitherto been advanced to show that dextrose and laevulose are precursors of the cane sugar in the leaf. We consider that in spite of the fact that it would be simpler, on theoretical or *a priori* grounds, to consider the hexoses as formed before the more complicated disaccharide, saccharose, the facts we have brought forward are better in accord with Brown and Morris' view that the cane sugar is the primary product of synthesis. It would seem, indeed, that plant leaves in general possess in *the chloroplasts a mechanism for elaborating cane sugar directly from the carbon dioxide of the air.* From the fact that cane sugar is the storage form in the sugar beet and mangold, the argument might be advanced, by those who regard dextrose as the primary product of synthesis, that the presence of cane sugar in the leaf of these plants is exceptional and due to there being here a special mechanism for its production. But we find that even in plants, like the potato (see p. 367), which store starch as a reserve substance, cane sugar is the predominating sugar in the leaf; and even in the grape (*Vitis vinifera*), where dextrose is the storage form, we find that, when special precautions are taken in sampling to prevent the leaf enzymes from inverting the saccharose present, the latter sugar is the principal sugar of the leaf[1].

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[1] This is quite contrary to Deleano's statement [1912] that cane sugar is not present in the vine leaf. Deleano's inability to detect saccharose was probably due to insufficient precautions being taken to prevent inversion.

The same is true of the snowdrop, studied by Parkin, which stores starch and inulin.

The relatively high proportion of cane sugar in the mangold leaf during the early stages of growth[1], as well as the experiments recorded by Parkin ([1912], p. 31) showing the enormous increase of the cane sugar which occurs in the leaf of the snowdrop when plants previously kept in darkness for 4 days are exposed to light, support the view that saccharose is actually a direct product of photosynthesis. In Parkin's experiments the cane sugar increased 2½ fold, whilst the hexoses only increased by one-third. It is probable that in the mesophyll of the leaf, saccharose is the *sole* sugar present, and the invert sugar is first formed in the veins or small vessels which serve as conducting channels to the mid-ribs. Our analyses of course could only be made with leaf tissue from which the largest conducting vessels were removed (viz. the mid-ribs), but the tissue actually worked with still contained all the smaller vessels and it was probably in these that the small proportion of reducing sugars found in the early stages of growth was located.

One of the most striking features of the stalks and mid-ribs is that the proportion of saccharose *remains practically constant throughout the whole 24 hours*, whilst the hexoses fluctuate between wide limits (see Figs. 7 to 10). Thus in Series I, for example, the cane sugar in the tops of the stalks varied only from 3·75 to 4·39 per cent., whilst the hexoses had a range of from 5·35 to 13·17 per cent. Moreover, whilst the percentages of reducing sugars in the top and bottom halves are widely different at any one time, the bottom half always being the richer, the proportion of saccharose in the two halves is practically the same. Thus, for example, at noon (Table V) when the invert sugar in the top half was 9·97 per cent. and in the bottom half 13·17 per cent., the cane sugar in the top half was 4·39 and in the bottom half 4·12 per cent. As the season advances the proportion of cane sugar at corresponding times of day changes but little, whereas the hexoses increase enormously. For example, we may take the following:

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[1] The predominance of cane sugar in the leaf in the early stages of growth points to the rate of production of this sugar exceeding the rate of its hydrolysis by invertase; the fact that during the morning the hexoses (see Fig. 4) increase faster than the cane sugar points to the hydrolytic effect of the enzyme increasing more rapidly with rise of temperature (or an actual increase in the amount of enzyme occurring) than the synthetic effect producing the saccharose. The parallelism of the curve showing the ratio $\frac{\text{I.S.}}{\text{C.S.}}$ with the temperature curve in both Series I and II is best explained in this way.

	August 26th, noon (average tops and bottoms)	Sept. 10th, 10 a.m.	Oct. 11th, 11 a.m.
Saccharose	4·25 %	4·82 %	5·29 %
Hexoses	11·57	20·5	25·7

It is a striking fact (compare Table V with Table VI and the data for mid-ribs and stalks in Table VIII) that the proportion of saccharose in the mid-ribs (which are nearer the leaf tissue) is slightly higher and somewhat less constant than in the stalks. For example, in Series II:

In *stalks*, saccharose varies from 4·82–5·26 per cent.; hexoses from 20·5–26·7 per cent.

In *mid-ribs*, saccharose varies from 6·08–7·44 per cent.; hexoses from 19·0–23·6 per cent.

On passing from the stalks, through the mid-ribs to the leaves, the range of variation of the saccharose during the day *increases*, but even in the leaves the variation of cane sugar *during the daytime* is far less marked than that of the hexoses. Parkin also observed in the snowdrop a similar phenomenon (*loc. cit.*, p. 28) as regards the seasonal variation, but the fluctuation of the hexoses between morning and evening was less marked than that of the saccharose (*loc. cit.*, p. 29). This was probably due to the fact that he took only two samples in the day, one at a time when the sugars were increasing, the other when they were falling. A similar false conclusion as to the approximate constancy of the hexoses and wide variation of the saccharose would be formed if samples had been taken in Series III (Fig. 6) at 9 a.m. and 3 p.m. only, but the general shape of the curves in Series I and II shows that during the *period of daylight* the hexose is the more variable even in the leaf.

The great variation during the day of the reducing sugars and the practical constancy of the cane sugar in the mid-ribs and stalks, and the fact that the proportion of cane sugar in the top and bottom halves of the stalks is practically the same, point to the relatively rapid movement or formation of the hexoses in the mid-ribs and stalks. In the case of the snowdrop, Parkin (*loc. cit.*, p. 25) found that the long, narrow *leaves* contained a far larger proportion of hexose to cane sugar in the upper parts than in the lower parts, especially when the plants were grown in clumps so that the lower parts were shaded; in this case, the lower halves of the leaves functioned mainly as stalks or media of translocation not as true assimilating leaves, the results presenting an exact parallel with those obtained in the case of the mangold stalks. Parkin also found that the *colourless* part of the snowdrop leaf which is enclosed by the membranous sheath is very rich in sugar—30 to 40 per cent. of its dry weight. On analogy with our results these lower portions of leaf correspond with the extreme lower parts of the mangold leaf-stalks, and would probably, if an analysis were made, be found to contain practically the whole of the sugar in the form of reducing sugar.

In the potato (see pp. 367–373), where saccharose is the predominating sugar in the leaf, the proportion of hexose in the stalks bearing the leaflets is far higher than in these leaflets (5 to 30 fold) whilst the saccharose is very nearly the same in both.

	Hexoses in		Saccharose in		Ratio $\frac{\text{I.S.}}{\text{C.S.}}$ in	
	Stalk	Leaflets	Stalk	Leaflets	Stalk	Leaflets
Minimum ...	4·63	0·15	2·65	1·76	0·10	1·54
Maximum ...	5·63	1·27	3·58	3·66	0·44	1·77

It would seem, therefore, that *in all plants of which a systematic examination has been made* (*mangold, sugar beet, potato, snowdrop, grape vine, dahlia, etc.*) *saccharose is formed directly in the mesophyll of the leaf, whence it passes into the veins, mid-ribs and stalks, undergoing more and more complete inversion in its passage.* The regulating mechanism is apparently such that a nearly constant concentration of cane sugar is maintained throughout the day and throughout the season in the mid-ribs and stalks, whilst the reducing sugars vary within very wide limits.

At first sight it would appear to be a clumsy and unnecessary contrivance for plants such as the mangold and sugar beet to form cane sugar in the leaf, then to transform it completely into hexoses in the stalks only to have to reconvert it back again into cane sugar in the roots; it would seem to be a simpler arrangement for the cane sugar to travel as such to the roots. As will be seen from the historical introduction given on pp. 255–262, most workers in this field have assumed this to be the case. It is a striking fact that even Girard's [1884] data show an enormous preponderance of the hexoses over the saccharose in the leaf-stalks of the sugar-beet (the hexoses being 5 to 10 times the cane sugar, which generally was small); but he was apparently so struck by the novel observation of the relatively large proportion of cane sugar in the leaf, that he quite ignored the significance of the stalk analyses, concluding that the saccharose passed *tout formé* from the leaf to the root.

It is probable however that the actual mechanism of storage adopted possesses certain well-defined advantages; in the first place, if the sugars travel by simple diffusion, as has frequently been assumed, the rate of diffusion of the reducing sugars would be four times that of the cane sugar; moreover, as was emphasised by Brasse [1886], if the storage of cane sugar is accomplished by a direct wandering of this sugar as such from the leaf to the root, it would be in direct defiance of the ordinary laws of diffusion, as motion would occur from a place of low concentration to one of high concentration. If, however, the sugar is translocated as hexose and is immediately transformed in the root into saccharose, these objections no longer hold and a continuous stream of sugar could be maintained. It is probable, too, that the actual mechanism adopted serves to keep the cane sugar, once it is formed in the root, from getting out; de Vries' [1879] well-known experiment may be recalled, in which he showed that strips of beet-root could be soaked in water for 14 days without the presence of sugar being detected in the surrounding water. Gutzeit [1911] has also recently emphasised the impermeability to saccharose of the protoplasm of the cell-walls of the beet-root, pointing out that in the ordinary process of manufacture of sugar from the beet it is necessary to use *hot* water first, to kill the protoplasm, before the sugar can be extracted. Brasse [1886] showed that the same effect could be produced by chloroform. If the protoplasm is permeable to the hexoses and not to the saccharose a simple mechanism would exist by which the root could store up sugar without any possibility of loss by back-diffusion, and an explanation would be given of the fact that although the *concentration* of sugar in the root may be diminished after heavy rains by the inflow of water, the actual *total quantity* of sugar in the root steadily increases throughout the season's growth and never shows any falling off (Vivien [1913]). It has been generally assumed that, in the *second* year's growth of the beet, when the cane sugar is utilised to form new shoots, the saccharose is first inverted by invertase in the root and is conveyed in the form of invert sugar to the growing points. But according to Colin [1915] invertase is not found in the *root* even in the second period of growth, when the seed-bearing plant is beginning to form; the saccharose is held to undergo inversion in the stalks and leaves of the new plant.

It still remains to consider the means by which the hexoses which are carried into the root are transformed into saccharose. The complete absence of invertase from the root militates against the view adopted by Robertson, Irvine and Dobson [1909] that the cane sugar is formed by a process of reversible zymo-hydrolysis, in which the invertase acts as a synthetic agent. These authors recognised this difficulty and were driven to assume that saccharose is formed, not in the root, but in the organs containing invertase—the leaves and stalks—and translocated as such. Our results prove that the sugars approaching the neighbourhood of the root become richer and richer in hexoses, and, as we shall shortly show, it is in the form of hexoses that the sugars actually enter the root. With regard to the theory that the cane sugar is formed by reversible enzyme action, the experiments quoted by Robertson, Irvine and Dobson to show a synthetic action of the enzyme-sludge prepared from parts of the sugar-beet are by no means conclusive; even if such synthetic action occurred (and its amount seemed to be exceedingly small) it is not shown that the synthetic action was due to invertase or that it was reversible. In all ordinary concentrations such as would be met with in the plant cells, invertase acts practically completely in the one direction only—that of hydrolysis. It would seem indeed that the root of the sugar beet or mangold possesses some special mechanism for synthesising cane sugar—some special enzyme such as the "saccharogenic enzyme" of Barbet [1896]. But there is as yet little direct evidence available in favour of such a theory.

That the reducing sugars conveyed by the stalks actually enter the root is shown by the recent analyses of Colin [1914], who states that when the root is exceedingly small (*souches filiformes*) the reducing sugars form one-fifth of the total sugars in the root. The *proportion* of reducing sugar naturally falls as the season advances, because the accumulation of the cane sugar stored diminishes the *relative* proportion of reducing sugar. But there is little doubt from the analyses we give in Table V that the reducing sugars, which more and more predominate in the stalks as the root is approached, actually enter the root as such throughout the season. Pellet [1914, 2], at the congress of sugar chemists in 1914, criticised the work of earlier workers, such as Girard, and pointed out that their failure to detect reducing sugars in the root was owing to their having used insufficiently delicate methods of analysis; he stated that reducing sugars are always present in the root in amounts varying from 0·05 to 0·30 per cent., depending on the meteorological conditions. When the hexoses are being rapidly formed and enter the root at a rate which is in excess of the ability of the "saccharifying power" of the root to cope with, the reducing sugars accumulate for a short time, and high values such as 0·3 per cent. are obtained. On other days, when the rate of production of the hexoses is less, the "saccharifying power" of the root is able immediately to transform the whole of the hexoses into saccharose, and lower values such as 0·02 to 0·03 per cent. are found.

Even in the typical saccharose-forming plant the sugar cane, M. Pellet informs us (private communication), reducing sugars are invariably present. In the early stages of growth (first 5 or 6 months) the cane may contain 3 per cent. of saccharose and 3 per cent. of hexoses; later the proportion becomes 5 per cent. of saccharose and 2 per cent. of hexoses, then 7 per cent. of saccharose and 1 per cent. of hexoses until finally the cane contains 12, 13 or even 16 per cent. of saccharose and only 0·6, 0·5, 0·2, or even 0·1 per cent. of reducing sugars[1]. The quantity of hexoses in the cane differs in different parts, increasing as one passes from the lower to the upper part of the stem. This, we consider, points to a steady influx of reducing sugars from the upper parts, followed by transformation and storage in the lower parts of the cane, which in this plant fulfil the functions of the root in the sugar beet and mangold.

Finally it will be well to emphasise the difference that exists between our views and those recently expressed by Pellet [1913] and by Colin [1914]. Pellet holds that saccharose, dextrose and laevulose are formed *simultaneously* (*tout à la fois*) in the leaves and that *all* these sugars pass into the root, which possesses the property of transforming the hexoses into saccharose. Colin expresses practically the same view – "la racine reçoive à la fois du saccharose qui s'emmagasine et du réducteur qui est polymerisé." We are quite at one with these workers that saccharose, dextrose and laevulose are *present* at the same time, but we consider that the cane sugar is probably first formed *alone* in the mesophyll of the leaf, that it is transformed into invert sugar by invertase in the veins and mid-ribs and finally more and more completely in its progress through the sieve-tubes of the stalks. It enters the root entirely in the form of reducing sugars and is therein reconverted into saccharose. Once in this form the sugar cannot escape, until it is put under contribution at the commencement of the following season's growth, for the building up of the new shoot.

Strakosch's views. Strakosch [1907] has put forward the view, based on micro-chemical tests, that in the mesophyll of the leaf only one sugar is present, namely, dextrose; laevulose is said first to occur in the small veins of the leaf and saccharose is formed as a final product in the veins and mid-ribs. It is in the form of saccharose that the sugar travels to the roots. Strakosch employed Grafe's [1905] micro-chemical test for laevulose, based on the use of methylphenylhydrazine; whether this test is at all characteristic of ketoses is still an open question. Strakosch and Neuberg [1904] consider that it is, but Ofner [1904 and 1905] and Ost [1905] regard it as unsatisfactory in presence of dextrose. With regard to Senft's [1904] test for dextrose which Strakosch relied upon it is perhaps sufficient to point out that Strakosch himself admits that "die Unterscheidung von Rohrzucker und Glucose nach dem Senftschen Verfahren ist an die richtige Schätzung des untersuchenden Auges gebunden[1]." Absolutely in contradiction with Strakosch's views, but in accord with our own, are the earlier micro-chemical observations of de Vries [1897], who found in the chlorophyll-containing cells of the sugar beet *no* reducing sugars at all, in the general tissue of the vascular bundles only small quantities, but in the larger vessels and veins increasing quantities of hexoses.

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[1] Pellet [1914, 1] shows that the reducing sugars found in the molasses of cane sugar manufacture represent the original hexoses of the juice and are not produced by inversion during the manufacturing operations.

Strakosch relies almost entirely on his micro-chemical tests. He quotes only two quantitative estimations of the sugars; in one, an extract of mesophyll tissue was found to contain 0·15 per cent. of hexoses and 0·026 per cent. of saccharose; in the other, an extract of vein tissue contained 0·12 per cent. of hexoses and 0·54 per cent. of saccharose. No details are given as to the preparation of these extracts, nor of the means taken to prevent enzyme changes. The results given absolutely contradict those obtained by Kayser [1883], Girard [1884], Ruhland [1911], Parkin [1912], Colin [1914] and ourselves, which show that in passing from the leaf tissue to mid-ribs and stalks, the proportion of hexoses to saccharose progressively and rapidly increases. If Strakosch's views were correct we should expect to find the hexoses in excess in the leaf tissue and the cane sugar steadily increasing on the way to the root. Actually the reverse is true. In the earliest stages of growth, saccharose predominates in the leaf and even in the later stages of growth *when the hexoses are in excess in the leaf, the proportion of cane sugar to reducing sugars is at its maximum in the leaf tissue and falls steadily in passing from the leaf to mid-ribs, and from mid-ribs to stalks.* If Strakosch's ideas were correct it is surprising to find, as the season advances and the importance of the storage function increases, the *proportion* of the reducing sugars to cane sugar in the stalks actually *increasing*; thus:

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[1] Since the above was written Mangham (*Annals Bot.* 1915, **29**, 369) has published some observations on the osazone method of locating sugars in plant tissues. As a result of his experiments he considers (p. 377) that any attempt to distinguish saccharose qualitatively in presence of its hexose constituents by Senft's method cannot give trustworthy results. Senft was led to attach too much importance to this method from the results obtained with 50 per cent. sugar solutions, and neglected to check them with weaker solutions comparable in concentration with the contents of plant cells. Mangham, therefore, like ourselves, considers that Strakosch's technique was unreliable and "only those of his conclusions with regard to cane sugar which were founded on evidence other than that derived in the above manner can be regarded as at all trustworthy." This would leave very little of the structure raised by Strakosch still standing.

It is difficult to understand Mangham's view that it is possible to discriminate between dextrose and laevulose by means of the osazone test, seeing that both sugars (as well as mannose) yield identically the same osazone: Mangham seems to regard the osazones from dextrose and laevulose as distinct substances.

In the writer's opinion little reliance can be placed on a micro-chemical osazone test as a means of identifying maltose in plant tissues, owing to the presence of large quantities of other sugars. Our quantitative analyses (some 500 in all) have in no single instance disclosed the presence of even traces of maltose in the leaves or conducting systems of plants. In work of this kind, micro-chemical tests as a means of distinguishing individual sugars should be avoided and only quantitative methods adopted. Otherwise contradictory and uncertain results are inevitable. [Note added, Dec. 9, 1915.] W. A. D.

	Stalks, Aug. 26th–27th (average top and bottom halves)	Stalks, Sept 10th–11th
Ratio $\frac{\text{I.S}}{\text{C.S.}}$ ...	ranges from 1·8 to 2·7	4·25–5·27
Hexoses ...	5·35–13·1 %	20·5–26·7 %

The following comparisons of the ratio of hexoses to saccharose for leaf, mid-ribs and stalks are incomprehensible on the basis of Strakosch's views.

	Leaf	Mid-ribs	Stalks
August 26th–27th ...	0·26–0·69	—	1·8 –2·7
September 10th–11th	1·11–1·60	2·55-3·72	4·25–5·27
October 11th–12th ...	1·14–1·93	2·75-3·24	3·99–4·86

We are aware that against the view that cane sugar is a primary product may be urged the fact that in Series I and II of our results the hexoses appear to increase after sunrise faster than the saccharose, so that they seem to be more responsive to the stimulus of light than the saccharose. But this is probably due to the fact that each molecule of saccharose gives rise on inversion to two molecules of hexose; when the separate proportions of dextrose and laevulose are considered (see following communication) they appear to follow the proportion of cane sugar more closely. The actual values found for cane sugar represent merely the excess of cane sugar formed over that inverted to hexoses; as we have already pointed out the proportion of hexoses to saccharose $\left(\frac{\text{I.S.}}{\text{C.S.}}\right)$ *during the daytime* (that is the period of photosynthetical action) follows very closely the temperature curve, as if the rate of inversion of the cane sugar increased with the temperature proportionately faster than the rate of formation of cane sugar itself.

We are also aware that it is possible to explain the predominance of saccharose in the early stages of growth (Series I), regarding the hexoses as primary products, by assuming that as the root is insufficiently developed to deal with them, they are transformed into the storage form, saccharose, in the leaf itself, so as to relieve the osmotic pressure. The formation of starch in the very early stages of growth, contrasted with its entire absence later on, is a similar phenomenon, having as its object the diminution of the excess sugar formation. But although this hypothesis is a possible one, it appears that the whole body of facts we have recorded, especially the data regarding translocation, find a better explanation in the view that the cane sugar is a primary product and gives rise to the hexoses by inversion than by assuming the hexoses to be primary products in the mesophyll and the saccharose to be formed from them.

SUMMARY.

1. The formation and translocation of the sugars in the mangold have been studied under actual conditions of growth, in which translocation was normal.

2. Starch is entirely absent from the leaf after the very earliest stages of growth. As soon as the root begins to develop so that the sugars formed in the leaf can be translocated to it, starch disappears almost entirely from the leaf. Maltose is entirely absent from leaf, mid-ribs and stalks at all stages of growth and at all times of night and day.

3. During the early stages of growth of the mangold, when leaf formation is the principal function, saccharose is present in the leaf tissue in excess of the hexoses. Later in the season, when sugar is being stored in the root, the reverse is true, hexoses largely predominating in the leaf.

4. In the mid-ribs and stalks the hexoses always predominate greatly over the saccharose and vary widely in amount during the day and night, and throughout the season, whilst the saccharose remains practically constant. In passing from leaves to mid-ribs, from mid-ribs to the tops of stalks and from the tops of stalks to the bottoms, the ratio of hexoses to saccharose steadily and rapidly increases. As the season advances the predominance of the hexoses in leaf, mid-ribs and stalks becomes more and more marked.

5. The proportion of saccharose in the leaf tissue follows the temperature curve closely during the daytime; the proportion of hexoses increases faster than the temperature, in such a way that the curve showing the ratio of hexoses to saccharose is itself practically parallel to the temperature curve.

6. The facts brought forward can apparently be best explained by Brown and Morris' view that saccharose is the primary sugar formed in the mesophyll of the leaf under the influence of the chlorophyll; it is transformed into hexoses for the purpose of translocation. This transformation occurs in the veins, mid-ribs and stalks, the proportion of hexoses increasing more and more as the root is approached. The sugar enters the root as hexose and is therein reconverted into saccharose; once in this form the saccharose is not able to leave the root until it is put under contribution for the second season's growth.

7. These views are in accord with de Vries' micro-chemical observations as to the nature of the sugars in the different tissues but entirely in contradiction with those of Strakosch, which are considered to rest on no secure foundation.

8. They also agree with Parkin's results with the snowdrop, with Pellet's analyses of the sugar cane, with Colin's results with the sugar beet and our own observations with other plants such as the vine (*Vitis vinifera*), potato, dahlia, etc., which store their carbohydrates in other forms (dextrose, starch and inulin).

9. As regards the mechanism by which saccharose is synthesised from the hexoses, it is improbable that this change is effected by invertase by a process of reversible zymo-hydrolysis. The entire absence of invertase from the root is against this view.

10. Pentoses form only a small proportion of the total sugars in the tissues; they are probably formed from the hexoses and appear to be precursors of the pentosans.

APPENDIX.

A. EXAMPLE SHOWING METHOD OF CALCULATING RESULTS.

Mangold Leaves. October 11th, 1912, 11 p m.

The alcoholic extract of the leaf material was evaporated *in vacuo* and made up to 500 cc.

20 cc. of the 500 cc. evaporated and dried *in vacuo* at 110° in the apparatus, Fig. 2, gave (*a*) 2·5833 grms.
(*b*) 2·5872 ,,

Average = 2·5852 grms. **64·63** grms. in 500 cc.

The leaf residue left after extracting the sugars, etc., weighed 75·31 grms. after drying at 100°. This still contained 6·84 % moisture, as determined by heating 10 grms. *in vacuo* at 110° (see p. 269).

∴ Weight of vacuum-dried matter in the 75·31 grms. = 75·31 × 0·9316
= **70·16** grms.

Total vacuum-dried matter in leaf sample = 64·63 + 70·16
= **134·79** grms.

$$\frac{\text{Matter extracted by alcohol}}{\text{Total vacuum-dried matter}} = \frac{64\cdot63}{134\cdot79} \times 100 = \mathbf{47\cdot9}\ \%.$$

FOR SUGARS.

440 cc. of the 500 cc. were precipitated by basic lead acetate (265 cc.), filtered and washed to 2 litres = *Solution A*.

300 cc. of *A* deprived of lead by solid sodium carbonate and made to 500 cc. = *Solution B.*

For reducing sugars.

25 cc. of *B* (= 15 cc. *A*) gave 0·2210 grm. CuO[1].

Rotation of Solution *B* in 200 mm. tube at 20° (Na flame) = + 0·274°.

For cane sugar.

50 cc. of Solution *B* were inverted by either citric acid or invertase. After inversion and neutralisation, made to 100 cc. = *Solution C′* (or *C″*)

(*a*) *Citric acid.*

50 cc. of *C* (= 25 cc. *B* = 15 cc. *A*) gave 0·3710 grm. CuO.

Solution *C′* gave rotation = − 0·064° in 200 mm. tube at 20°.

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[1] In all cases the value of the cupric reducing power given is the average of two or three closely concordant results. The separate determinations always agreed to within 1 to 2 mgs. of CuO.

(*b*) *Invertase.*

50 cc. of *C′* (= 15 cc. *A*) gave 0·3706 grm. CuO.
Solution *C′* gave rotation = − 0·071° in 200 mm. tube at 20°.

Maltose.

300 cc. of Solution *A* deprived of lead by hydrogen sulphide were diluted to 500 cc. = *Solution B′.*

Three 50 cc. portions of Solution *B′* were fermented by *S. exiguus*, *S. marxianus* and *S. anomalus.* After treatment with 5 cc. of alumina cream each solution was made to 100 cc.; 50 cc. of the filtrate (= 15 cc. *A*) gave 0·0134 grm. CuO (average of the three).

Two 50 cc. portions were fermented similarly with distillery yeast. 50 cc. of the final solution (= 15 cc. *A*) gave 0·0127 grm. CuO (average of the two).

Pentoses.

50 cc. *A* gave 0·0149 grm. phloroglucide.

Pentose $= (0\cdot0149 + 0\cdot0052) \times 1\cdot017 \times \frac{2000}{50} \times \frac{500}{440} \times \frac{100}{134\cdot79} = \mathbf{0\cdot68}\ \%$ on the T.V.D.M.

CALCULATION OF RESULTS.

Saccharose. (*a*) *Citric acid.*

From reduction. Increase of reduction in 25 cc. *B* (= 15 cc. *A*) caused by inversion

$$0\cdot3710 - 0\cdot2210 = 0\cdot1500 \text{ grm. CuO.}$$

This corresponds, using the factor for 0·3710 grm. of CuO in Brown, Morris and Millar's Tables, to $\frac{0\cdot1500}{2\cdot354}$ grms. of invert sugar.

% of saccharose on T.V.D.M. of leaf

$$= \frac{0\cdot1500}{2\cdot354} \times 0\cdot95 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134\cdot79} = \mathbf{6\cdot80}\ \%.$$

(*b*) *Invertase.* Increase of reduction on inversion of 15 cc. *A*

$$= 0\cdot3706 - 0\cdot2210 = 0\cdot1496 \text{ grm. CuO.}$$

∴ % of saccharose (as in (*a*)) = **6·78** %.

From Polarisation data. (*a*) *Citric acid.*

Change of rotation of *B* in 200 mm. tube caused by inversion

$$= 0{\cdot}274° + (2 \times 0{\cdot}064°) = 0{\cdot}402°.$$

$$\% \text{ of saccharose} = \frac{0{\cdot}402}{1{\cdot}744^{*}} \times \frac{500}{100} \times \frac{2000}{300} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{6{\cdot}48}\ \%.$$

(*b*) *Invertase.* Change of rotation of *B*

$$= 0{\cdot}274° + (2 \times 0{\cdot}071°) = 0{\cdot}416°.$$

$$\therefore\ \% \text{ of saccharose (as in } (a)) = \mathbf{6{\cdot}70}\ \%.$$

Apparent Dextrose and Laevulose.

Reduction of 25 cc. *B* (= 15 cc. *A*) = 0·2210 grm.

Reduction due to pentoses in 25 cc. *B*

$$= \frac{15}{50} \times 0{\cdot}0201 \times 1{\cdot}017 \times 2{\cdot}65^{\dagger} = 0{\cdot}0163 \text{ CuO}.$$

$$\therefore \text{ Reduction due to hexoses} = \mathbf{0{\cdot}2047} \text{ grm. CuO}.$$

Grms. pentose in 100 cc. *B*

$$= 4 \times \frac{15}{50} \times 0{\cdot}0201 \times 1{\cdot}017 = 0{\cdot}0245 \text{ grm}.$$

Rotation of pentose in *B* in 200 mm. tube.
If *arabinose* = 102·2 × 200 × 0·0245 = **0·050°**.
If *xylose* = 18·8 × 200 × 0·0245 = **0·009°**.
Now the concentration of saccharose in 100 cc. *B*

$$= \frac{6{\cdot}79}{100} \times 134{\cdot}79 \times \frac{440}{500} \times \frac{3}{5} \times \frac{1}{20} = 0{\cdot}2417 \text{ grm}.$$

Rotation due to saccharose in *B* (200 mm. tube)

$$= \frac{66{\cdot}44 \times 200 \times 0{\cdot}2417}{10^4} = \mathbf{0{\cdot}321}°.$$

∴ Rotation due to hexoses in *B*,

calculating pentoses as arabinose = 0·274 − 0·050 − 0·321° = − **0·097°**,

" " " xylose = 0·274 − 0·009 − 0·321° = − **0·056°**.

*** The factor 1·744 represents the change of rotation in a 200 mm. tube caused by the inversion of a 1 per cent. solution of saccharose.**

† Assuming the pentoses to be a mixture of equal quantities of arabinose and xylose and using the tables given by Daish [1914] for the reducing power of the pentoses. The value 0·0163 found is slightly higher than the residual reduction 0·0130 after fermentation, which is due to the pentoses.

Proportions of Dextrose and Laevulose.

(A) *Pentoses as arabinose.* If x = grms. of dextrose in 25 cc. *B*, y = grms. of laevulose in 25 cc. *B*,

we have[1]

$$4{\cdot}22x - 7{\cdot}36y = -0{\cdot}097^\circ,$$
$$2{\cdot}56x + 2{\cdot}34y = 0{\cdot}2047.$$

Solving for x and y,

$x = 0{\cdot}04457$ grm. dextrose in 25 cc. *B* (= 15 cc. *A*),
$y = 0{\cdot}03872$ grm. laevulose in 25 cc. *B* (= 15 cc. *A*).

$$\therefore \text{ Dextrose in T.V.D.M.} = 0{\cdot}04457 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{5{\cdot}01}\ \%.$$

$$\text{Laevulose in T.V.D.M.} = 0{\cdot}03872 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{4{\cdot}35}\ \%.$$

$$\therefore \text{ D} + \text{L} = \mathbf{9{\cdot}36}\ \%.$$

$$\frac{\text{D}}{\text{L}} = \frac{5{\cdot}01}{4{\cdot}35} = \mathbf{1{\cdot}151}.$$

(B) *Pentoses as xylose.*

$$4{\cdot}22x - 7{\cdot}36y = -0{\cdot}056^\circ,$$
$$2{\cdot}56x + 2{\cdot}34y = 0{\cdot}2047.$$

Hence as in (A),

% of dextrose = **5·39** %
% of laevulose = **3·94** %
D + L = **9·33** %

$$\frac{\text{D}}{\text{L}} = \frac{5{\cdot}39}{3{\cdot}94} = \mathbf{1{\cdot}37}.$$

Calculating Reducing Sugars as Invert Sugar.

$$\text{Invert sugar in 25 cc. } B = \frac{0{\cdot}2047}{2{\cdot}445} = 0{\cdot}08351 \text{ grm.}$$

$$= 0{\cdot}08351 \times \frac{2000}{15} \times \frac{500}{440} \times \frac{100}{134{\cdot}79} = \mathbf{9{\cdot}39}\ \% \text{ on T.V.D.M.}$$

If the rotation in *B* were due to invert sugar, in 200 mm. tube at 20°, rotation

$$= \frac{0{\cdot}08351 \times 4 \times 200 \times -19{\cdot}6}{10} = \mathbf{0{\cdot}131^\circ}.$$

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[1] The constants 4·22 and 7·36 for dextrose and laevulose are calculated from the specific rotatory powers $[\alpha]_D^{20^\circ} = 52{\cdot}7$ and $[\alpha]_D^{20} = -92{\cdot}0^\circ$ for 1 per cent. solutions of the sugars. The constants 2·56 and 2·34, corresponding with the 0·2210 grm. CuO actually weighed, are taken from Brown, Morris and Millar's Tables for dextrose and laevulose.

Instead of actually observed, pentoses as arabinose = − **0·094°**,
and actually observed, pentoses as xylose = − **0·053°**.

Maltose. Reduction corresponding with 15 cc. *A*.

After fermentation with maltase-free yeasts = 0·0134 grm. CuO
After fermentation with distillery yeast = 0·0127
Δ = 0·0007 grm. CuO

The difference between the two sets of fermentations is within the experimental error of the method.

∴ Maltose = **0·00** %.

Starch. Exp. 1. 10·449 grms. of oven-dried leaf matter after extracting sugars gave, on drying *in vacuo* at 110°, 9·7355 grms.

∴ Moisture = **6·82** %.

The leaf material was heated with 200 cc. of water in boiling water for 15 minutes and, after cooling, treated with 0·1 grm. taka-diastase at 38° for 24 hours. After boiling to destroy the enzymes, the leaf material was filtered on a Buchner funnel and washed with water until the washings amounted to about 450 cc. The filtrate was transferred to a 500 cc. measuring flask and exactly the necessary quantity of basic lead acetate solution (8·5 cc.) added to precipitate the tannins, etc. The solution was then, without filtering, made up to 500 cc. at 15°. After filtering, 100 cc. of the filtrate were treated with solid sodium carbonate to precipitate the lead present and made up to 110 cc. After filtering, 50 cc. were used to estimate the reducing power.

Weight of CuO = 0·0000 grms.
Polarisation in 400 mm. tube = − 0·053°.

The entire absence of reducing power shows the absence of maltose and dextrose and hence of starch in the original material. The laevo-rotation is due to the gummy matter of the leaf which is not entirely precipitated by the basic lead acetate, owing to the slight solubility of its lead compound (Davis and Daish [1914]).

Exp. 2. Gave Moisture = 6·86 %,
Starch = 0·00 %.

B. EXPERIMENTAL DATA.

Series I. Mangold Leaves. August 26th–27th, 1913.

The alcoholic extract was evaporated *in vacuo* and made up to 500 cc. 440 cc. of this solution were treated with basic lead acetate and the filtrate and washings deprived of lead by adding solid sodium carbonate: the solution was then diluted to 2000 cc. = *A*.

Time	Dry matter sol in alcohol (vacuum-dried), grms.	Dry matter insol in alcohol (vacuum-dried), grms.	Total vacuum-dried matter (T.V.D.M.), grms.	Direct reducing power of 25 cc. *A*. grms. CuO	Polarisation of *A* in 200 mm. tube, $\alpha_D^{20°}$	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*. grms.
						Reduction of 25 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube*	Reduction of 25 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube*	
6 a.m.	49·78	67·42	117·20	0·0370	+0·279°	0·1068	−0·001°	0·1094	−0·001	0·0043
8 a.m.	54·85	74·27	129·12	0·0647	+0·215	0·1659	−0·063	0·1662	−0·061	0·0065
10 a.m.	56·45	81·76	138·21	0·0978	+0·250	0·2172	−0·044	0·2168	−0·049	0·0078
12 noon	52·00	76·25	128·25	0·0895	+0·240	0·2000	−0·034	0·2049	−0·039	0·0062
2 p.m.	51·57	86·66	138·23	0·0882	+0·266	0·2044	−0·075	0·2147	−0·085	0·0070
4 p.m.	49·60	83·70	133·30	0·0584	+0·322	0·1726	±0·000	0·1756	±0·000	0·0068
6 p.m.	55·47	82·90	138·37	0·0538	+0·267	0·1594	−0·014	0·1704	−0·044	0·0082
8 p.m.	47·31	80·40	127·71	0·0444	+0·247	0·1243	−0·003	0·1338	−0·006	0·0048
10 p.m.	54·00	79·60	133·60	0·0412	+0·183	0·1200	−0·010	0·1250	−0·012	0·0056
12 night	43·72	71·48	115·20	0·0320	+0·186	0·0959	−0·011	0·1103	0·011	0·0058
2 a.m.	44·19	70·25	114·44	0·0252	+0·157	0·0720	−0·008	0·0803	−0·002	0·0048
4 a.m.	39·70	64·72	104·42	0·0213	+0·107	0·0590	+0·005	0·0718	+0·003	0·0066

* This polarisation refers to the diluted solution after inversion, the dilution being twice that of *A*.

Mangold Stalks. August 26th 27th, 1913.

The extract was concentrated *in vacuo* and made up to 100 cc., 70 cc. of this being treated with basic lead acetate and washed to nearly 500 cc. The excess of lead was removed by solid Na_2CO_3 and solution made to 500 cc. = *A*.

Time		Dry matter sol in alcohol (vacuum-dried), grms.	Dry matter insol in alcohol (vacuum-dried), grms.	Total vacuum-dried matter (T.V.D.M.), grms.	Direct reducing power of 25 cc. *A*, grms. CuO	Polarisation of *A* in 200 mm. tube, α_D^{20}	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*, grms.
							Reduction of 25 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube	Reduction of 25 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm. tube	
6 a.m.	Tops ...	10·17	9·06	19·23	0·1114	+0·302°	0·1836	+0·044°	0·1700	+0·084°	0·0123
6 a.m.	Bottoms	15·45	13·00	28·45	0·2533	+0·556	0·3501	+0·153	0·3398	+0·153	0·0183
Noon.	Tops ...	11·07	9·10	20·17	0·1893	+0·100	0·2660	+0·040	0·2706	+0·048	0·0071
Noon.	Bottoms	16·22	11·64	27·86	0·3440	+0·557	0·4412	+0·136	0·4093	+0·177	0·0235
6 p.m.	Tops ...	12·79	11·05	23·84	0·1880	+0·187	0·2714	+0·072	0·2760	+0·071	0·0142
6 p.m.	Bottoms	19·86	14·51	34·37	0·2745	+0·654	0·3721	+0·085	0·3707	+0·090	0·0233
12 night.	Tops ...	9·51	9·29	18·80	0·1260	+0·282	0·1936	+0·024	0·1960	+0·019	0·0094
12 night.	Bottoms	14·68	11·63	26·31	0·2172	+0·448	0·3138	+0·122	0·2958	+0·144	0·0140

Series II. Mangold Leaves. September 10th–11th, 1912.

(1) Extract evaporated *in vacuo* and made to 250 cc. 200 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre. Excess of lead removed by solid sodium carbonate and made to 1000 cc = *A*.

Time	Dry matter sol. in alcohol (vacuum-dried), grms	Dry matter insol in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms	Direct reducing power of 5 cc. *A*, grms. CuO	Polarisation of *A* in 200 mm. tube, $\alpha_D^{20^\circ}$	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*, grms.
						Reduction of 5 cc. *A* after inversion, grms CuO	α_D after inversion in 200 mm. tube	Reduction of 5 cc. *A* after inversion, grms. CuO	α_D after inversion in 200 mm tube	
10 a.m.	127·92	105·75	233·67	0·1380	+0·754°	0·2444	−0·325°	0·2486	−0·183°	0·0265

(2) Extract evaporated *in vacuo* and made to 250 cc. 200 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre = *A*. 375 cc. of *A* were deprived of lead by solid Na_2CO_3 and made up to 500 cc. *B*. For reduction used 10 cc. *B* 7·5 cc. *A*. (Solution after inversion *C*.)

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol. in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms.	Direct reducing power of 7·5 cc. *A*, grms. CuO	Polarisation of *B* in 200 mm. tube, $\alpha_D^{20^\circ}$	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *B*, grms.
						Reduction of 7·5 cc. *A* after inversion, grms. CuO	α_D after inversion in *C* (200 mm.)	Reduction of 7·5 cc. *A* after inversion, grms. CuO	α_D after inversion in *C* (200 mm.)	
1 p.m.	62·50	78·92	141·42	0·1655	+0·381°	0·2635	−0·228°	0·2730	−0·270°	0·0110

(3) Extract evaporated *in vacuo* and made up to 500 cc. 440 cc.[1] of the 500 treated with basic lead acetate, filtered and washed to nearly

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[1] In some cases, indicated in Table under "Remarks," 460 cc. were used instead of 440 cc.

2 litres. Solid sodium carbonate added and solution made up to 2000 cc. = *A*.

Time	Dry matter sol. in alcohol (vacuum-dried), grms.	Dry matter insol in alcohol (vacuum-dried), grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (*x*)	Polarisation of *A* in 200 mm. tube $\alpha_D^{20°}$	Reduction of *x* cc. of *A*, grms. CuO	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*	Remarks
							Reduction of *x* cc. *A* after inversion, grms. CuO	α_D after inversion (200 mm.)	Reduction of *x* cc. *A* after inversion	α_D after inversion (200 mm.)		
4 p.m.	68·20	72·36	140·56	20 cc.	+0·215°	0·2434	0·3820	−0·107°	0·3840	−0·118°	0·0163	460 cc.
11 p.m.	67·60	68·08	135·68	20 cc.	+0·390	0·2294	0·4203	−0·160	0·4172	−0·165	0·0157	440 cc.
2 a.m.	66·88	65·10	131·98	15 cc.	+0·470	0·1862	0·3643	−0·235	0·3650	−0·195	0·0165	440 cc.
4 a.m.	80·50	76·40	156·90	15 cc	+0·350	0·1926	0·3373	−0·200	0·3400	−0·220	0·0159	440 cc.
6 a.m.	62·10	68·66	130·76	25 cc.	+0·170	0·2445	0·3945	−0·154	0·3940	−0·143	0·0129	440 cc.
8 a.m.	61·51	73·15	134·66	20 cc.	+0·235	0·1753	0·3075	−0·147	0·3185	−0·144	0·0126	440 cc.

(4) Extract evaporated *in vacuo* and made up to 500 cc. 440 cc. (or 460 cc.) of the 500 treated with basic lead, filtered and washed to 2 litres = *A*. 30 cc. of *A* were precipitated with solid sodium carbonate to remove the lead and made up to 500 cc. = *B*. 25 cc. of *B* used for direct reduction.

Time	Dry matter sol in alcohol grms.	Dry matter insol. in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube α_D^{20}	Reduction of *x* cc. of *B*, grms. CuO	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*	Remarks
							Reduction of *x* cc. *B* after inversion, grms. CuO	α_D after inversion (200 mm.)	Reduction of *x* cc. *B* after inversion	α_D after inversion (200 mm.)		
6 p.m.	75·38	72·52	147·90	25 cc.	+0·135°	0·2333	0·3920	−0·162	0·3955	−0·228°	0·0098	460 cc.
8 p.m.	64·34	71·89	136·23	25 cc.	+0·130	0·1662	0·2863	−0·165	0·2938	−0·124	0·0161	440 cc.

Mangold Stalks. September 10th–11th, 1912.

Extract evaporated *in vacuo* and made up to 200 cc. 170 cc. (or 180 cc.) of the 200 treated with basic lead acetate, filtered and washed to nearly 2 litres. Solid sodium carbonate added to precipitate the lead exactly and solution made to 2000 cc. = *A*.

Time	Dry matter sol. in alcohol. grms.	Dry matter insol. in alcohol, grms.	Total vacuum-dried matter. grms.	Volume of solution *A* used for reduction (x)	Polarisation of *A* in 200 mm. tube $\alpha_D^{20^\circ}$	Reduction of x cc. of solution *A*, grms. CuO	Invertase inversion: Reduction of x cc. *A* after inversion. grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. *A* after inversion grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
10 a.m.	67·20	35·57	102·77	10 cc.	+0·636°	0·2279	0·2767	+0·228°	0·2862	+0·097°	0·0186	170 cc.
4 p.m.	43·86	24·69	68·55	20 cc.	+0·380	0·3828	0·4505	+0·021	0·4639	+0·069	0·0117	180 cc.
11 p.m.	38·44	20·32	58·76	20 cc.	+0·471	0·2836	—	—	0·3481	+0·055	0·0079	170 cc.
4 a.m.	52·64	29·29	81·93	15 cc.	+0·595	0·3288	0·3982	+0·090	0·4012	+0·124	0·0134	180 cc.
6 a.m.*	43·30	23·96	67·26	25 cc. *B*	+0·277	0·3045	0·3595	+0·062	0·3637	+0·048	0·0113	180 cc.

* In the 6 a.m. analysis, 300 cc. of *A* were treated with solid sodium carbonate and made up to 500 cc. (=*B*). The polarimetric data therefore refer to solution *B*.

Mangold Mid-ribs. September 10th–11th, 1912.

Extract evaporated *in vacuo* and made up to 100 cc. 80 cc. of the 100 cc. treated with basic lead acetate, filtered and washed to 500 cc. = Solution *A*. 150 cc. of Solution *A* deprived of lead by solid sodium carbonate and made up to 200 cc. = Solution *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol. in alcohol, grms.	Total vacuum-dried matter. grms.	Volume of solution *B* used for reduction (x)	Polarisation of *B* in 200 mm. tube $\alpha_D^{20^\circ}$	Reduction of x cc. of solution *B*, grms. CuO	Invertase inversion: Reduction of x cc. *B* after inversion, grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. *B* after inversion, grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*
10 a.m.	17·65	11·35	29·00	15 cc.	+0·636°	0·3025	0·3825	−0·028°	0·3858	−0·041°	0·0061
4 p.m.	12·43	7·36	19·79	20 cc.	+0·520	0·2715	0·3380	—	0·3489	+0·076	0·0101
11 p.m.	10·15	7·27	17·42	25 cc.	+0·382	0·2766	0·3618	—	0·3646	+0·006	0·0102
4 a.m.	15·05	8·16	23·21	25 cc.	+0·425	0·3385	0·4631	+0·042	0·4625	+0·059	0·0177
6 a.m.	12·90	8·50	21·40	25 cc.	+0·367	0·3446	0·4437	—	0·4455	+0·007	0·0130

Series III. Mangold Leaves. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 500 cc. 440 cc. of the 500 were treated with basic lead acetate, and the precipitate washed until the volume of filtrate was exactly 2 litres = *A*. 300 cc. (or 250 cc.)* of the solution were precipitated by solid sodium carbonate and made up to 500 cc. = Solution *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol in alcohol, grms.	Total vacuum-dried matter. grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube α_D^{20}	Reduction of *x* cc. of solution *B*, grms. CuO	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*	Remarks
							Reduction of *x* cc. after inversion, grms. CuO	α_D after inversion (200 mm.)	Reduction of *x* cc. after inversion, grms. CuO	α_D after inversion (200 mm.)		
9 a.m.	65·97	60·42	126·39	25 cc. *B*	+0·212°	0·2289	0·3410	−0·084°	0·3469	−0·097°	0·0172	300 cc. *A*
11 a.m.	81·80	67·88	149·68	25 cc. *B*	+0·217	0·2524	0·3943	−0·132	0·3997	−0·119	0·0242	250 cc. *A*
1 p.m.	75·00	67·72	142·72	25 cc. *B*	+0·113	0·2503	0·3825	−0·152	0·3892	−0·141	0·0233	250 cc. *A*
3 p.m.†	97·94	80·34	178·28	10 cc. *A*	+0·690	0·2145	0·3906	−0·344	0·3870	−0·273	0·0281	*A* direct
5 p.m.	76·95	69·48	146·43	25 cc. *B*	+0·189	0·2427	0·3897	−0·141	0·3941	−0·165	0·0221	250 cc. *A*
7 p.m.	92·74	79·09	171·83	25 cc. *B*	+0·304	0·2286	0·4058	−0·131	0·4048	−0·125	0·0288	200 cc. *A*
9 p.m.	73·53	62·23	135·76	25 cc. *B*	+0·194	0·2345	0·3670	−0·098	0·3715	−0·100	0·0194	250 cc. *A*
11 p.m.	64·63	70·16	134·79	25 cc. *B*	+0·274	0·2210	0·3706	−0·071	0·3710	−0·064	0·0149	300 cc. *A*
1 a.m.	73·71	66·34	140·05	25 cc. *B*	+0·230	0·2614	0·4120	−0·157	0·4225	−0·153	0·0192	300 cc. *A*
3 a.m.	76·25	60·25	136·50	25 cc. *B*	+0·263	0·2868	0·4688	−0·168	0·4683	−0·171	0·0156	300 cc. *A*
5 a.m.	74·72	61·92	136·64	25 cc. *B*	+0·279	0·2679	0·4190	−0·121	0·4202	−0·125	0·0155	300 cc. *A*
7 a.m.†	66·36	62·36	128·72	20 cc. *A*	+0·234	0·2818	0·4165	−0·198	0·4220	−0·188	0·0119	*A* direct

* Whether 250 cc. or 300 cc. of the 500 cc. were used is shown in the last column. At 7 p.m. 200 cc. of *A* were taken.

† In these analyses the solution *A* was used direct, without further dilution, so that the polarimetric and reduction data refer to the solution *A*.

Mangold Stalks. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 200 cc. 170 cc. of the 200 precipitated by basic lead acetate, filtered and washed to 2 litres = Solution *A*. 300 cc. of *A* treated with solid sodium carbonate and made up to 500 cc. = *B*.

Time	Vacuum-dried matter sol. in alcohol. grms.	Vacuum-dried matter insol. in alcohol. grms.	Total vacuum-dried matter, grms.	Volume of solution *B* used for reduction (*x*)	Polarisation of *B* in 200 mm. tube $\alpha_D^{20^\circ}$	Reduction of *x* cc. of solution *B*, grms. CuO	Invertase inversion		Citric inversion		Phloroglucide from 50 cc. *A*	Remarks
							Reduction of *x* cc. after inversion, grms. CuO	α_D after inversion (200 mm.)	Reduction of *x* cc. after inversion, grms. CuO	α_D after inversion (200 mm.)		
11 a.m.	43·70	25·67	69·37	25 cc. *B*	+0·332°	0·2865	0·3417	+0·045°	0·3483	+0·040°	0·0095	300 cc. *A*
11 p.m.*	40·16	25·56	65·72	25 cc. *A*	+0·492	0·3735	0·4605	+0·102	0·4663	+0·093	0·0110	*A* used direct

* Solution *A* used direct without further dilution, so that polarimetric and reduction data refer to solution *A*.

Mangold Mid-ribs. October 11th–12th, 1912.

Extract evaporated *in vacuo* and made up to 100 cc. 70 cc. of the 100 cc. precipitated by basic lead acetate, filtered and washed to 1 litre = Solution *A*. 300 cc. of *A* precipitated by solid Na_2CO_3 and made up to 500 cc. = *B*.

Time	Vacuum-dried matter sol. in alcohol, grms.	Vacuum-dried matter insol. in alcohol, grms.	Total vacuum-dried matter. grms.	Volume of solution *B* used for reduction (x)	Polarisation of *B* in 200 mm tube $\alpha_D^{20^\circ}$	Reduction of x cc. *B*, grms. CuO	Citric inversion		Phloroglucide from 50 cc. *A*	Remarks
							Reduction of x cc. after inversion, grms. CuO	α_D after inversion (200 mm.)		
11 a.m.	10·56	5·95	16·51	25 cc. *B*	+0·100	0·1032	0·1343	−0·007	0·0056	300 cc. *A* to 500
11 p.m.*	9·07	6·15	15·26	25 cc. *A*	+0·214	0·1503	0·2032	+0·006	0·0039	*A* used direct

* In the 11 p.m. analysis 80 cc. (instead of 70 cc.) of original 100 cc were taken; the solution *A* was treated with solid sodium carbonate and used direct without further dilution.

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(*Received October* 4, 1915.)

STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

II. THE DEXTROSE-LAEVULOSE RATIO IN THE MANGOLD

By WILLIAM A. DAVIS.

(Rothamsted Experimental Station.)

Brown and Morris [1893] in their well-known experiments on the *Tropæolum* leaf observed that the hexoses of the leaf instead of being present in the proportion corresponding with invert sugar, invariably appeared to consist very largely of laevulose. In several cases dextrose was entirely absent, whilst in others the proportion of laevulose to dextrose varied from about 6 : 1 down to about 2 : 1. As they had concluded on other grounds that the reducing sugars are formed by inversion from cane sugar, they explained the predominance of laevulose as being due to the dextrose being "more readily put under contribution for the respiratory processes of the cell than is laevulose."

Lindet (1900) made a special study of the proportion of the hexoses present in the leaf and leaf-stalks of the sugar beet at different periods of growth. His analyses showed that in normally growing leaves, especially in the earlier stages of growth (July 3rd to 24th), the proportion of dextrose was generally slightly *greater* than that of laevulose, although on several occasions it was slightly *less*; thus on July 3rd and July 24th, for example, it was found that the ratio of laevulose to dextrose was 1·3 and 1·11 respectively. On the other hand and in striking contrast to the leaves, the laevulose in the leaf-stalks was *invariably* found to form only a small proportion of the dextrose present, varying from 0 to 35 per cent. Lindet adopts Brown and Morris' views to explain these results and concludes that the excess of laevulose in the leaves is due to the dextrose being consumed in these tissues by *respiration* more rapidly than the laevulose; on the other hand the laevulose has been removed from the sap of the stalks in forming new tissue, laevulose being the sugar specially adapted to this purpose. Lindet in a recent paper [1911], inspired by the results of his earlier work, cites experiments made with yeasts which also serve to show that laevulose lends itself to reproductive growth or new tissue formation better than dextrose.

Parkin [1912] in experiments on the snowdrop leaf found that in 47 out of 52 analyses the laevulose was in excess of the dextrose; representing laevulose as unity, in these cases the ratio varied from 1 : 0·4 to 1 : 0·76. In the five remaining cases, the ratio was 1 : 1·01 to 1 : 1·06. The ratio of laevulose to dextrose appeared to rise during the night, that is when photosynthesis is in abeyance; the excess of laevulose was always greatest in the lower (colourless) part of the long snowdrop leaf. To explain his results Parkin also adopted Brown and Morris' view that the dextrose lends itself most readily to the respiratory needs of the plant, whilst the laevulose is used largely in constructive work such as the building up of the plant's framework.

As was pointed out by Brown and Morris in 1893 the correctness of the dextrose and laevulose values depends entirely on the accuracy of the readings of the rotatory power; a slight error in these makes a large difference in the apparent proportion of the two hexoses. The main purpose of the present paper is to show that, whilst it is possible to take the actual readings very accurately (in our case the probable error did not exceed 0·005°), the values are falsified, in the case of most plant material, by the presence of optically active substances other than the sugars, which are not completely precipitated by the basic lead acetate (or other defecating substance) used to purify the solutions. Typical substances of this kind are amino-acids and amides, such as glutamic acid and glutamine, aspartic acid and asparagine. The first three of these have a pronounced positive rotation which is greatly enhanced by acids, whilst asparagine is laevo-rotatory in aqueous solution and dextro-rotatory in acid solution. The influence of these substances in falsifying the results obtained by the method of double polarisation for the cane sugar in the molasses of sugar manufacture has been studied by several chemists, more particularly by H. Pellet (compare *Dosage du Sucre par Inversion*, 1913), but the effect of these and other impurities on the results obtained for the dextrose : laevulose ratio in plant material has not hitherto been taken into account.

We find in the *mangold*, just as Lindet did in the sugar beet, that the dextrose always seems to be in excess of the laevulose, especially in the mid-ribs and stalks (where the ratio $\frac{D}{L}$ is often extremely high), whereas in the *potato* the reverse holds, the laevulose apparently predominating as in the cases studied by Brown and Morris, and by Parkin. At the same time it can be shown that the apparent excess of dextrose or laevulose is correlated with certain abnormalities in the cane sugar estimations, caused by the presence of optically active impurities. The apparent excess of *dextrose* in the tissues of certain plants (sugar beet and mangold) is indeed due to the presence of a dextro-rotatory impurity (possibly glutamine), whilst the predominance of laevulose in other plants (e.g. tropæolum, snowdrop, potato) is to be attributed to a laevo-rotatory impurity (e.g. asparagine).

In the mangold the difference between the results obtained for saccharose by the reduction method and by the double polarisation method, which we have referred to in the preceding paper (p. 273), is always far greater in the stalks and mid-ribs than in the leaves, a fact which we attribute to the accumulation of optically active impurities in these parts. Side by side with this we have the fact that, whilst in the leaves the ratio of dextrose to laevulose $\left(\frac{D}{L}\right)$ is in general not very far removed from unity, in the stalks and mid-ribs the ratio $\frac{D}{L}$ is very much greater, generally varying from 2·5 to 10. This ratio, too, is far higher in the bottom halves of the stalks than in the top halves, pointing to an accumulation in the lower part of the stalks of the dextro-rotatory impurity. Striking differences are also found between the results for cane sugar in the top and bottom halves, according as they are calculated from the reduction data or from the polarimetric values. Thus in the *top* halves the results obtained by polarisation may be 80 to 90 per cent. *lower* than the values obtained by reduction, whilst in the *bottom* halves they are *high* by 40 per cent. As the day proceeds, the relation of tops and bottoms may be reversed, the impurity which was in the top half passing down to the lower part of the stalks (compare the values at noon, 6 p.m. and midnight given on p. 344, Table VIII).

Independently of any error which may be caused by the improper use of basic lead acetate (see p. 270), a difficulty which makes it impossible to obtain really accurate values of the proportion of dextrose and laevulose lies in the fact that allowance has to be made in the calculation for the reducing power and rotation of the pentoses which are invariably present in the alcoholic extracts prepared by our method of working.

In any particular case, one is entirely ignorant as to the nature of these; if it be assumed that they consist of arabinose and xylose, whilst it is possible to introduce a fairly accurate correction for the reducing power, owing to the fact that the reducing power of arabinose is nearly identical with that of xylose (Daish [1914]), this is not the case for the rotatory power as $[\alpha]_D$ has widely different values for the two pentoses (for arabinose $[\alpha]_D^{20} = +122°$, for xylose $[\alpha]_D^{20°} = +18{\cdot}78°$). This large disparity in the specific rotatory powers may, in certain cases, involve a difference of 0·1° or more in the rotation from which the dextrose and laevulose are calculated, according as the pentose is assumed to be arabinose or xylose respectively; in the example given, showing our method of calculation (see p. 317), the difference is only 0·011°, but it is frequently much greater and then represents quite a large proportion of the actual angle used in calculating the reducing sugars. On this account, in default of information as to the exact nature of the pentoses present, we have always calculated the dextrose and laevulose on the two assumptions: (1) that the pentose is arabinose, (2) that the pentose is xylose. But it is quite possible that the pentose really present may consist to a greater or less extent of one of the less known pentoses, e.g. *d*-ribose, and if this is the case the results for dextrose and laevulose will be correspondingly at fault.

Dextrose and laevulose are, moreover, calculated from values obtained after allowing for all the other substances present—cane sugar, pentoses, maltose (if present). The degree of accuracy obtained will naturally depend on the accuracy with which the other constituents have been estimated. Even the difference caused by calculating the small proportion of pentose as arabinose or as xylose may, as for example in the mangold leaf, 5 p.m., October 11th, make the ratio $\frac{D}{L}$, which appears to be strictly 1·00 when the pentose is taken as xylose, have a very different value (0·844) when the pentose is assumed to be arabinose.

In putting forward the results given in this paper, I am conscious that the values given as dextrose and laevulose probably do not, in most cases, represent real values; they are therefore designated "apparent dextrose" and "apparent laevulose." Although little confidence can be placed in them as *absolute* values for these sugars, they show a regular variation which is sufficiently striking to justify detailed consideration. This variation may be due either to a real variation of the dextrose and laevulose or, what is more probable, to a regular variation in the amount of the optically active impurities which are present; if the latter, the fluctuation of these substances during the 24 hours must be quite as great as the fluctuation of the sugars themselves. Until a method has been devised by which it is possible to estimate accurately the true proportions of dextrose and laevulose, when present together, without having recourse to polarimetric data, the facts brought forward in this paper, and that which follows, show that it is impossible to know with any certainty the real proportions of these sugars present in different plant tissues; it is, therefore, equally impossible to draw conclusions as to the function of these two sugars in the plant—whether the one is more suited than the other to build up new tissue or whether one is more easily put under contribution than the other in respiration. The fermentation test, which Parkin and others have used to ascertain whether the solutions they analysed were free from optically active substances other than sugars, is one which is by no means reliable for this purpose. Parkin, whose work in most other respects is valuable, considered that, as the solutions prepared from the snowdrop leaves showed, after fermentation with yeast, a negligible rotatory and reducing power, *no other substances likely to possess these properties were present in the original solutions.* It would be quite possible for large amounts of asparagine to have been present, sufficiently large indeed to explain the apparent preponderance of laevulose in the snowdrop (where the ratio of laevulose to dextrose varied from 1 : 0·4 to 1 : 0·76) and yet to have escaped detection by this method, as the asparagine would be largely, if not entirely, consumed by the yeast in its growth; asparagine indeed is used very largely as a nutrient material for yeasts, for example in Hayduck's solution.

EXPERIMENTAL.

The methods of analysis have been described in the preceding paper; an example is there given of the method of calculating the proportions of dextrose and laevulose according as the pentoses are assumed to be arabinose or xylose (see p. 318). The actual data used are given on pp. 319–325; the results are calculated on the *total vacuum-dried matter* of the leaf.

In the tables which follow, D = per cent. of "apparent dextrose" in the total vacuum-dried matter; L = per cent. of "apparent laevulose" in the total vacuum-dried matter.

TABLE I.

Mangold Leaves. Series I. August 26th–27th, 1913.

Time	Pentose as arabinose			Pentose as xylose			*D*+*L* %, pentose taken as		Hexoses calculated as invert sugar %	
	D %	*L* %	$\frac{D}{L}$	*D* %	*L* %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m. ...	0·73	Nil	∞	0·73	Nil	∞	0·73	0·73	0·77	
8 a.m. ...	0·64	0·77	0·83	0·87	0·52	1·68	1·41	1·39	1·42	
10 a.m. ...	1·09	1·06	1·02	1·32	0·80	1·65	2·15	2·12	2·16	
12 noon ...	1·11	1·02	1·08	1·33	0·78	1·70	2·13	2·11	2·15	Day
2 p.m. ...	1·01	0·90	1·12	1·24	0·66	1·88	1·91	1·90	1·94	
4 p.m. ...	0·92	0·21	4·34	1·15	0·00	∞	1·13	1·15	1·18	
6 p.m. ...	0·49	0·46	1·07	0·74	0·19	3·87	0·95	0·93	0·96	Sun sets 7 p.m.
8 p.m. ...	0·73	0·14	5·33	0·92	0·00	∞	0·87	0·92	0·90	
10 p.m. ...	0·29	0·45	0·65	0·50	0·23	2·17	0·74	0·73	0·74	
12 night ...	0·32	0·24	1·35	0·55	0·00	∞	0·56	0·55	0·57	Night
2 a.m. ...	0·29	0·08	3·62	0·36	0·00	∞	0·37	0·36	0·38	
4 a.m. ...	0·00	0·20	0·00	0·16	0·02	7·14	0·20	0·19	0·20	Sun rises 5.5 a.m.

TABLE II.

Mangold Leaves. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			*D*+*L* %, pentose as		Hexoses calculated as invert sugar %	
	D %	*L* %	$\frac{D}{L}$	*D* %	*L* %	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	2·72	3·01	0·904	2·91	2·80	1·039	5·73	5·71	5·72	
1 p.m. ...	3·85	3·62	1·063	4·06	3·37	1·204	7·47	7·43	7·50	Day
4 p.m. ...	3·11	3·87	0·804	3·47	3·46	1·003	6·98	6·93	7·00	
6 p.m. ...	3·59	5·38	0·668	3·83	5·11	0·749	8 97	8·94	8·90	Sun sets 6.26 p.m.
8 p.m. ...	2·56	4·25	0·602	2·95	3·74	0·789	6·81	6·69	6·76	
11 p.m. ...	3·34	3·79	0·881	3·73	3·36	1·110	7·13	7·09	7·10	Night
2 a.m. ...	3·43	4·39	0·781	3·84	3·93	0·977	7·82	7·77	7·81	
4 a.m. ...	3·17	3·73	0·851	3·51	3·36	1·045	6·90	6·87	6·91	Sun rises 5.30 a.m.
6 a.m. ...	2·64	3·68	0·717	2·99	3·30	0·906	6·32	6·29	6·30	Day
8 a.m. ...	2·26	3·15	0·718	2·60	2·78	0·935	5·41	5·38	5·38	

TABLE III.

Mangold Leaves. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
9 a.m.	5·62	4·67	1·203	6·07	4·18	1·450	10·29	10·25	10·32	Day
11 a.m.	5·32	6·35	0·839	5·82	5·81	1·001	11·67	11·63	11·62	
1 p.m.	5·17	7·03	0·735	5·67	6·49	0·874	12·20	12·16	12·12	
3 p.m.	4·79	5·43	0·882	5·26	4·91	1·070	10·22	10·17	10·24	
5 p.m.	5·24	6·21	0·844	5·72	5·69	1·005	11·45	11·41	11·46	Sun sets 5.15 p.m
7 p.m.	5·67	5·82	0·975	6·17	5·27	1·171	11·49	11·44	11·47	Night
9 p.m	5·92	6·04	0·980	6·38	5·54	1·151	11·96	11·92	11·98	
11 p.m.	5·04	4·32	1·165	5·41	3·91	1·385	9·36	9·32	9·39	
1 a.m	5·25	5·53	0·950	5·69	5·04	1·129	10·78	10·73	10·78	
3 a.m	6·05	6·37	0·950	6·44	5·95	1·084	12·42	12·39	12·41	
5 a.m.	6·26	5·15	1·215	6·64	4·73	1·404	11·41	11·37	11·49	Sun rises 6.21 a m.
7 a.m.	4·81	4·80	1·002	5·15	4·44	1·160	9·61	9·59	9·62	Day

TABLE IV.

Mangold Leaf-stalks. Series I. Top and Bottom Halves.
August 26th–27th, 1913.

Time		Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %	
		D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose		
6 a.m.	Tops ...	4·36	0·76	5·72	5·09	0·00	∞	5·12	5·09	5·35	Day
	Bottoms	7·90	0·94	8·37	8·55	0·23	37·2	8·84	8·78	9·11	
12 noon	Tops ...	4·89	5·04	0·97	5·37	4·51	1·19	9·93	9·88	9·97	
	Bottoms	10·20	2·70	3·77	11·00	1·83	6·01	12·90	12·83	13·17	
6 p.m.	Tops ...	4·22	3·61	1·17	4·87	2·89	1·68	7·83	7·76	7·89	
	Bottoms	8·45	1·71	4·95	9·10	1·00	9·10	10·16	10·20	10·47	Sun sets 7 p.m.
12 night	Tops ...	5·09	1·29	3·95	5·71	0·61	9·35	6·38	6·32	6·61	Night
	Bottoms	6·80	1·37	4·97	7·38	0·73	10·05	8·17	8·11	8·49	

TABLE V.

Mangold Leaf Mid-ribs. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%, pentose as		Hexoses calculated as invert sugar %	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	17·8	5·3	3·36	18·0	5·1	3·53	23·1	23·1	23·6	Day
4 p.m. ...	17·9	4·1	4·37	18·4	3·5	5·26	22·0	21·9	22·6	
11 p.m. ...	15·3	4·9	3·12	15·8	4·3	3·68	20·2	20·1	20·6	Night
4 a.m. ...	12·9	5·8	2·22	13·6	5·1	2·67	18·7	18·7	19·0	
6 a.m. ...	14·4	6·7	2·15	15·2	5·9	2·58	21·1	21·1	21·4	Day

TABLE VI.

Mangold Stalks. Series II. September 10th–11th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%, pentose as		Hexoses calculated as invert sugar %	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Arabinose	Xylose		
10 a.m. ...	14·8	5·1	2·90	15·3	4·6	3·33	19·9	19·9	20·5	Day
4 p.m. ...	17·5	8·45	2·07	18·1	7·75	2·34	25·9	25·9	26·3	
11 p.m. ...	17·3	4·5	3·84	17·8	3·9	4·56	21·8	21·7	22·4	Night
4 a.m. ...	17·3	6·0	2·88	17·8	5·4	3·30	23·3	23·2	23·7	
6 a.m. ...	19·1	7·6	2·51	19·75	6·95	2·84	26·7	26·7	26·7	Day

TABLE VII.

Mangold Stalks and Mid-ribs. Series III. October 11th–12th, 1912.

Time	Pentose as arabinose			Pentose as xylose			$D+L$ %, pentose as		Hexoses calculated as invert sugar %
	D %	L %	$\frac{D}{L}$	D %	L %	$\frac{D}{L}$	Arabinose	Xylose	
Mid-ribs:									
11 a.m. ...	15·7	7·2	2·18	16·1	6·7	2·40	22 9	22·8	23·4
11 p.m. ...	14·0	4·5	3·11	14·5	3·9	3·73	18·5	18·4	19·0
Stalks:									
11 a.m. ...	19·3	5·8	3·32	19·9	5·2	3·81	25·1	25·1	25·7
11 p.m. ...	16·15	4·85	3·33	16·8	4·2	4·04	21·0	21·1	21·4

DISCUSSION OF RESULTS.

A. *Leaves.*

Series I. In Series I, Table I, the actual percentages of total hexoses are very small, especially at night, during which they range from 0·90 to 0·20 per cent.; consequently, even the small differences in the rotation of the pentoses, according as they are assumed to be arabinose or xylose, lead to considerable differences in the proportions of dextrose and laevulose apparently present. Thus, for example, at 8 a.m., if the pentose is assumed to be arabinose, laevulose appears to be in excess of the dextrose, the ratio $\frac{D}{L}$ being 0·83; if the pentose be taken as xylose, the dextrose appears in excess, the ratio $\frac{D}{L}$ becoming 1·68. In this particular case the rotation of the pentose represented, in a 200 mm. tube at 20°, as arabinose a reading of $+0·049°$, as xylose $+0·009°$, whilst, after allowing for the saccharose and pentoses present (see method of calculation, p. 317), the rotation left for the hexoses was $-0·042°$ or $-0·002°$ in the two cases.

That dextro-rotatory impurities are present in this series to an extent sufficient to invalidate the calculations and so to convey a false idea of the true quantities of dextrose and laevulose, is shown by considering the data obtained at 6 a.m. In this case, if the *whole* of the reducing sugar, obtained from the reduction data, is calculated as dextrose, a rotation + 0·039° (in 200 mm. tube at 20°) is obtained, whereas the rotation actually observed, after allowing for the cane sugar and pentoses, was + 0·093° (pentoses as arabinose) or + 0·125° (pentoses as xylose). Thus in the two cases a *positive* rotation remains unaccounted for, of + 0·054° or + 0·086° respectively. Similarly at 4 p.m., the whole of the reducing sugars calculated as dextrose would barely account for the actual positive rotation observed if the pentoses be taken as xylose, the excess being + 0·004°; if, however, the pentoses be taken as arabinose, the dextrose would more than account for the rotation observed by + 0·037°, and in this case the dextrose becomes 0·92 per cent. and laevulose 0·21 per cent., the ratio $\frac{D}{L}$ being 4·34. Similarly at 8 p.m. the assumption that the whole of the reducing sugar is dextrose leaves + 0·008° unaccounted for if the pentose is xylose; when it is taken as arabinose, D becomes 0·73 and L – 0·14 per cent., the ratio $\frac{D}{L}$ being high, viz. 5·33. Similar observations hold for midnight, 2 a.m. and 4 a.m.; at 4 a.m. the quantity of reducing sugars is so small, that assuming the pentose to be arabinose causes the laevulose to appear in excess, whilst if it is taken as xylose, the dextrose appears largely in excess, $\frac{D}{L}$ being 7·14.

It is clear therefore that little significance can be attached to the values for dextrose and laevulose in Series I owing to the presence of a dextro-rotatory impurity[1], the rotation of which is large relatively to that of the small quantity of sugars present. Even though this is the case, between 8 a.m. and 2 p.m. the values of dextrose and laevulose are approximately equal, especially when the pentoses are assumed to be arabinose, the ratio $\frac{D}{L}$ being approximately 1. It should be noted that at these times the proportion of the sugars is greatest, so that the effect of the rotation of the optically active impurity in falsifying the results is least marked. At night, apparently, an excess of this impurity is liberated, possibly as a waste product of metabolism, owing to degradation changes predominating, so that the laevulose appears to have disappeared entirely at 6 a.m., and a relatively large *positive* rotation remains unaccounted for even when the whole of the reducing sugar is assumed to be dextrose.

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[1] A determination was made for us by Mr E. Horton of the amino-nitrogen in the Solution A used in estimating the sugars in the case of a sample of mangold leaves picked at 2.45 p.m. on October 8th, 1914. 2 c.c. of this solution gave in the van Slyke micro-apparatus 0·225 c.c. of nitrogen at 0 and 760 representing 0·007 grm. of amino-nitrogen per 100 c.c. Calculated as *glutamine* this would represent 1·2 per cent. of glutamine on the total vacuum-dried weight of the leaf at a time of day when, judging by the results of Table III, the proportion of such impurity is at its minimum; in this picking, the cane sugar was 7·5 per cent. and the hexoses 19·1 per cent. of the total vacuum-dried matter, so that the proportion of the optically active amide is in this case only small relatively to the sugars, a fact which would explain that the ratio $\frac{D}{L}$ keeps in the neighbourhood of unity (see Tables II and III).

Fig. 1 shows the variation of "apparent dextrose" and "apparent laevulose" on the assumption that the pentoses are xylose[1] during the 24 hours, August 26th–27th. Throughout this period the dextrose curve is above the laevulose; during the period of actual insolation the curves are approximately parallel to each other and to the saccharose curve. If it were not for the presence of the dextro-rotatory impurity, the two curves would probably nearly coincide—the dextrose curve being lowered and the laevulose curve being correspondingly raised. On the assumption that the pentose is arabinose, the two curves actually coincide without any such correction being made. The parallelism of the curves of dextrose and laevulose with that of cane sugar is particularly striking when the apparent steepness of the curve of total hexoses is taken into account (see previous paper, Fig. 4), from which it might

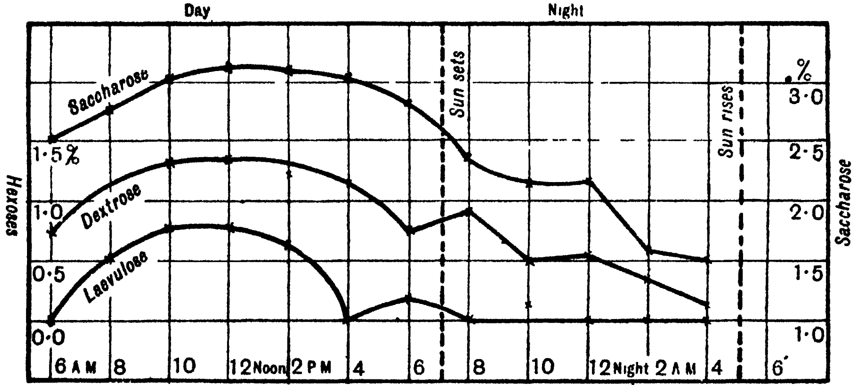

Fig. 1. Mangold leaves, apparent dextrose and laevulose, Series I, Aug. 26–27, 1913 (pentose as xylose).

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[1] Throughout this paper the curves of dextrose and laevulose are drawn only for the case when the pentose is assumed to be xylose. The curves obtained by assuming the pentose to be arabinose are strictly parallel to these curves but slightly higher or lower; the effect of taking the pentose as arabinose instead of xylose is to raise the laevulose curve and lower the dextrose curve.

be inferred that the hexoses are formed more rapidly than the saccharose, and consequently precede it; instead, the quantity of each of the reducing sugars is roughly proportional to the cane sugar present, a fact which points to the formation of the hexoses from this sugar.

Series II. In Series II (Table II) the sugar percentages are far higher than in Series I and the fluctuations in the value $\frac{D}{L}$ are far less marked in consequence. With few exceptions (e.g. at 6 p.m. and 8 p.m.) the value of $\frac{D}{L}$ does not differ much from 1, the percentages of dextrose and laevulose being as nearly equal as one could expect bearing in mind the errors to which the calculations are subject. In general, the values of $\frac{D}{L}$ obtained by assuming the pentose to be arabinose are slightly *lower* than unity, whilst by assuming it to be xylose, they become slightly *higher* than unity. It is probable that $\frac{D}{L}$ would be almost exactly 1 were it not for the presence of small quantities of optically active impurities, which in some cases increase the value, in others lower it. The following table shows that the departure of the value $\frac{D}{L}$ from 1 goes hand in hand with the divergence Δ between the values for cane sugar found by the reduction and double polarisation methods; this divergence is no doubt also caused by the presence of these optically active impurities (see p. 344).

$\frac{D}{L}$ approximately 1

Time	$\frac{D}{L}$	Δ*
10 a.m. ...	1·039	−14·0 %
4 p.m. ...	1·003	+ 1·5
11 p.m. ...	1·110	+ 7·2
2 a.m. ...	0·977	+ 7·4
4 a.m. ...	1·045	+16·1
6 a.m. ...	0·906	+10·0
8 a.m. ...	0·935	+10·6

$\frac{D}{L}$ divergent from 1

Time	$\frac{D}{L}$	Δ
1 p.m. ...	1·204	+25·9 %
6 p.m. ...	0·749	+15·2
8 p.m. ...	0·789	+23·2

* Δ represents the difference between the *reduction* and *polarisation* values for *cane sugar*, expressed as a percentage of the *average* value found by reduction by the invertase and citric acid methods; thus, e.g., +10·0 per cent. shows that the average value found by the double polarisation method is 10 per cent. higher than the average value found by reduction. In this particular case (6 a.m.) the cane sugar found by reduction was 4·24 per cent. on T.V.D.M., and by double polarisation 4·65 per cent.

The closer the value $\frac{D}{L}$ approximates to 1, the closer is the agreement between the cane sugar values by the two methods; at 4 p.m. particularly, when the value $\frac{D}{L}$ is practically 1, the divergence Δ is exceedingly small, viz. 1·5 per cent. only. It would appear that at this time the amount of optically active impurities influencing the results is practically *nil.* On the other hand, at 1 p.m., 6 p.m., and 8 p.m., when the value of $\frac{D}{L}$ departs considerably from unity, *in either direction*, there is a correspondingly large difference in the results for cane sugar by the two methods, the polarisation results being from 15 to 25 per cent. high. The figures at 6 p.m. and 8 p.m. are particularly interesting because at these times there is apparently an excess of laevulose[1], pointing to the presence of a *laevo-rotatory*, not a dextro-rotatory, impurity such as was present in the earlier part of the day (e.g. at 1 p.m.). At the same time, however, the polarisation figures are still higher than the reduction figures, showing that the *change* of rotation which accompanies the inversion process involves probably transformation of the laevo-rotatory substance into a compound with a still greater laevo-rotation—such as would happen, for example, in the transformation of asparagine into aspartic acid.

It is interesting to consider the curves in Fig. 2 showing the variation of the "apparent dextrose" and "apparent laevulose" during the 24 hours. Although these curves probably do not show the variation of the true sugars so much as that of the optically active impurities, they exhibit a regularity which points to the latter substances being formed regularly and progressively. In general the apparent dextrose and apparent laevulose increase when the cane sugar increases and fall when this sugar falls. The dextrose curve rises and falls three times in succession during the 24 hours, the rise and fall in the night (8 p.m. to 8 a.m.) being far more gradual and regular than the two abrupt changes during the day. The laevulose curve is less regular, rising gradually till 4 p.m., when D and L are practically equal; from this point the laevulose rises suddenly and follows the abrupt rise in the saccharose curve, which takes place just before sunset. The latter rise is therefore associated with a sudden increase in the amount of a *laevo-rotatory* impurity; at 4 p.m. the laevulose curve rises above the dextrose curve and remains above it until 10 p.m. From 6 p.m. to 8 p.m. $\frac{D}{L}$ has abnormally low values (0·749–0·789), but at 10 p.m. it is again practically unity, and it so remains throughout the night with very little change, although the laevulose curve rises and falls between 11 p.m. and 4 a.m., and falls between 4 a.m. and 8 a.m.

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[1] This apparent excess of laevulose would formerly have been explained by assuming that at these times the respiratory changes (utilising dextrose) predominate over the tissue-building changes. The polarisation data clearly point to abnormal quantities of optically active impurities at these points, which falsify the real values of D and L.

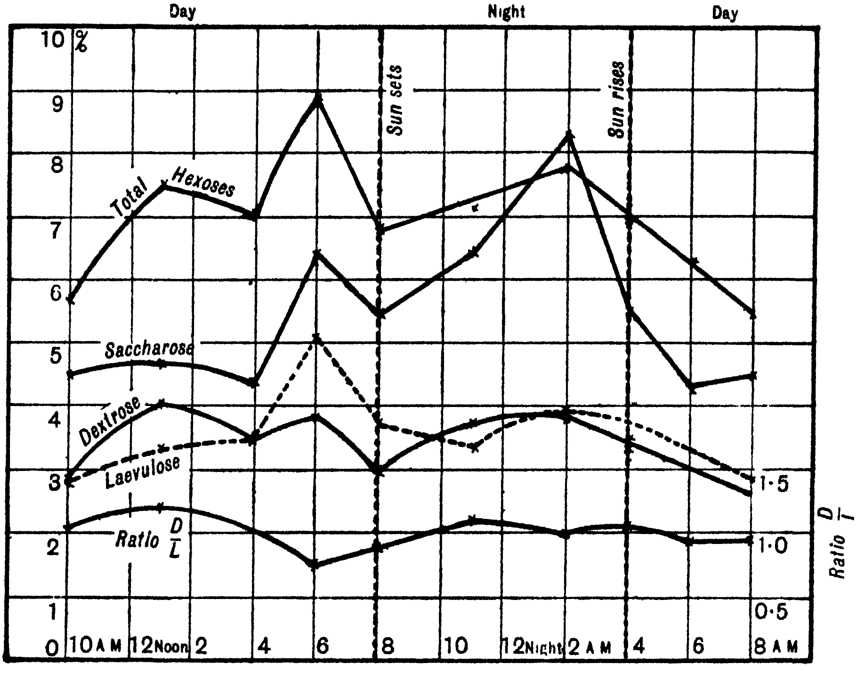

Fig. 2. Apparent dextrose and laevulose, Series II, Mangold leaves, Sept. 10–11, 1912.

The curve showing the variation of the ratio $\frac{D}{L}$ is also given in Fig. 2; it illustrates the marked periodic character of the fluctuations. These occur in two well-defined periods: In the first, a regular rise and fall of the ratio occurs in the eight hours from 10 a.m. to 6 p.m., during which the dextrose appears in excess; in the second period, from 6 p.m. to 10 p.m., the laevulose is in excess, but the ratio $\frac{D}{L}$ increasing. In the remaining 12 hours, from 10 p.m. to 10 a.m. there is little change in the value $\frac{D}{L}$ which remains very nearly unity.

Series III (Table III, Fig. 3). As in Series II, the ratio $\frac{D}{L}$ is, with few exceptions (9 a.m., 11 p.m. and 5 a.m.), approximately unity when the pentoses are taken as xylose; in most cases the ratio is greater than 1 when the pentoses are assumed to be xylose and less than 1 when they are taken as arabinose. As in Series II it can be shown that when the ratio $\frac{D}{L}$ most departs from unity (5 a.m., 7 a.m., 9 a.m.) the difference

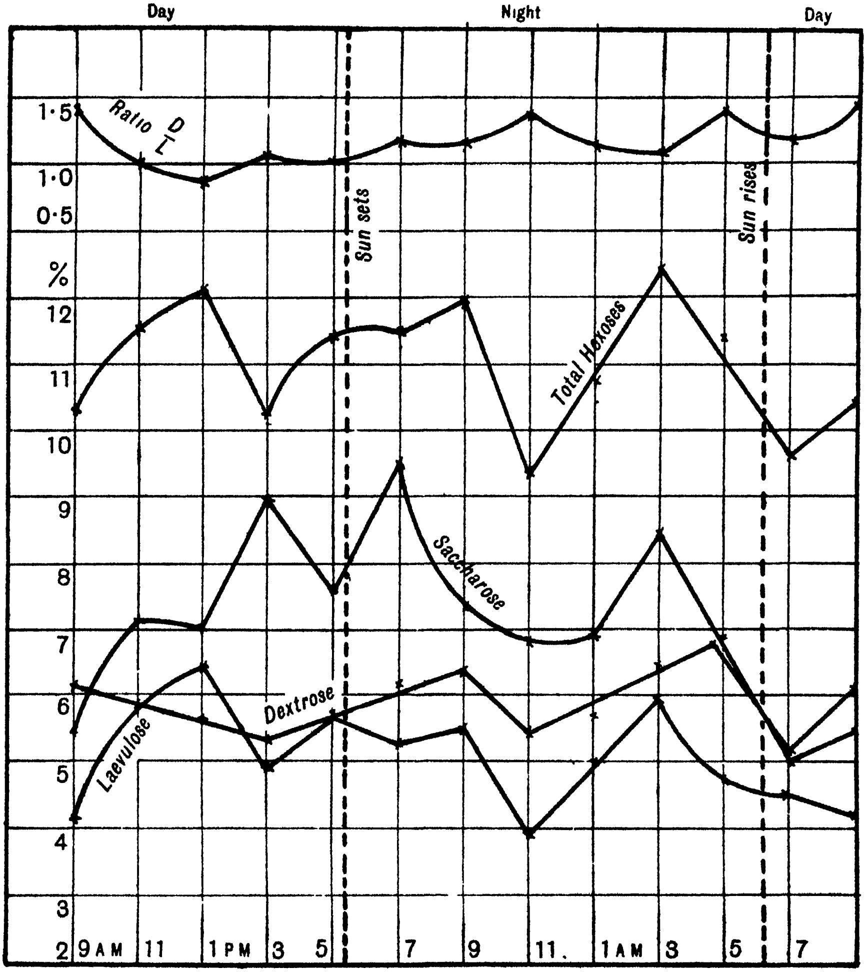

Fig 3. **Apparent dextrose and laevulose, Mangold leaves, Series III, Oct. 11–12, 1912.**

Δ between the values found for saccharose by reduction and polarisation is greatest, the polarisation figures being 20 to 30 per cent. high.

During the greater part of the day (except at 9 a.m., when $\frac{D}{L} = 1{\cdot}45$) the proportions of apparent dextrose and laevulose are very nearly equal, as might be expected if they were formed from saccharose, but at night the dextrose appears to be in excess. A striking difference from Series II is that the dextrose instead of rising during the day and then falling, at first appears to *fall* and then to rise (see Fig. 3); at night, instead of a rise and fall, there is a fall between 9 and 11 p.m., followed by a gradual rise to 5 a.m. But in both series there appears to be a similar periodic character in so far as there are three rises and three falls in the 24 hours. In Series III the variation of the apparent dextrose is most striking as taking place along practically straight lines in a very regular manner. The apparent laevulose curve in Series III is entirely different from the dextrose curve but it follows fairly closely the curve of total hexoses, and less closely the curve of saccharose. The fluctuations of the apparent laevulose are considerably greater and more abrupt than those of the dextrose, as was also the case in Series II.

The curve showing the variation of $\frac{D}{L}$ is also given. $\frac{D}{L}$ falls from 9 a.m. to 1 p.m., then rises slowly and more or less by successive steps to a maximum at 11 p.m., when it again falls and rises twice before 9 a.m.

B. *Stalks and Mid-ribs.*

The most striking fact which appears from the data given in Tables IV to VII is that in the stalks and mid-ribs the apparent dextrose is always in large excess of the laevulose. Whereas in the *leaf* the ratio $\frac{D}{L}$ does not depart much from unity[1], in the mid-ribs and stalks it is rare to find this ratio anywhere in the neighbourhood of 1. Only in the earliest stages of growth (August 26th) and then only in the top half of the stalks, nearest the leaf, at noon and 6 p.m., when freshly formed sugars from the leaf are passing into the stalks, does the ratio

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[1] The departure of this ratio from unity in Series I, Table I, is to be attributed to the impossibility, owing to the presence of optically active impurities, of ascertaining the true proportions of dextrose and laevulose in these cases, where the reducing sugars are present in small amounts. In Series II and III, the ratio $\frac{D}{L}$ is nearly unity throughout the whole 24 hours.

$\frac{D}{L}$ become nearly equal to unity. In all other cases the ratio varies from 2·5 to 5, or even higher in the case of the bottom halves of the stalks. In passing from the leaves to mid-ribs, from mid-ribs to the tops of stalks and from the tops of stalks to the bottom the proportion of apparent dextrose steadily and rapidly increases.

Series I. Tops and bottoms of stalks. The analyses given in Table IV show that the proportion of apparent dextrose to laevulose $\left(\frac{D}{L}\right)$ is far higher in the bottom half of the stalks than in the top half at the same time. At 6 a.m. of August 26th at first sight it would appear that during the night the laevulose practically disappears from both top and bottom halves of the stalk just as it appeared to do in the leaf (see Table I) during the night in the same series. Lindet observed a similar phenomenon in the case of the sugar beet and attributed the predominance of dextrose in the stalks to the laevulose being used more rapidly than the dextrose for purposes of tissue building. But that it is quite unsafe to rely upon the polarimetric data as affording any real index of the proportions of dextrose and laevulose actually present in the stalks is shown by the following considerations. The stalks stand out in striking contrast to the leaves as regards the extraordinary divergences between the results obtained for saccharose by the reduction method and by the method of double polarisation. In some cases, for example at 6 p.m., Table IV, the polarisation results for cane sugar in the bottom halves are 40 per cent. *higher* than the values obtained by reduction; and yet *at the same time* the tops give polarisation results which are 85 per cent. *low*. The following table (Table VIII) gives a comparison of the data obtained for cane sugar by the two methods (reduction and polarisation), showing that the divergence is very much greater in the stalks than in the leaves and much more variable in its nature. The fact that the *tops* may give by polarisation a large apparent deficiency of saccharose and the *bottoms* at the same time a large excess as compared with the reduction values (see the data at 12 noon and 6 p.m., Table VIII), or *vice versa* as at midnight when the relations are reversed, points to the presence *in the top and bottom halves of the stalk at different periods of the day of quite different impurities. with different and opposite rotatory powers* (substances as different as *d*- and *l*-glutamine or *d*- and *l*-asparagine).

A careful comparison of Table VIII with the curves showing the variation of apparent dextrose and laevulose in the stalks (Figs. 4

and 5) shows that *it is possible to correlate the wide variations in the differences* (Δ) *between the reduction and polarisation values for cane sugar with the variations in the apparent dextrose and laevulose*, a fact which points to their having a common origin, namely the presence of optically active impurities. Fig. 4 shows the variation of the apparent dextrose and laevulose in the *top* half of the stalks and also the curves for cane sugar and total hexoses. During the whole 24 hours the "dextrose" fluctuates only slightly—there is a slight rise and fall during the day and a slight rise and fall at night. The saccharose also is nearly constant during the 24 hours. But the "apparent laevulose" varies enormously. Between 6 a.m. and noon this sugar increases from *nil* to 4·5 per cent., but from noon onwards falls along almost a straight line until the zero is again reached shortly after midnight. The important fact to be noted is that *while the apparent laevulose increases the differences* (Δ) *between the polarisation and reduction values of cane sugar become more and more negative* (change from + 10 to − 90 for invertase values, Table VIII), whilst when the apparent laevulose falls the values of Δ become more and more positive (− 84 per cent. at 6 p.m., + 28 at midnight). It may be noted that the curve of apparent

Table VIII.

Divergence of Results for Saccharose by Reduction and Polarisation in Mangold Stalks—Series I.

		Saccharose found				Polarisation results *high* by % (Δ)	
		Citric inversion		Invertase inversion			
		By reduction	By polarisation	By reduction	By polarisation	Citric inversion	Invertase inversion
6 a.m.	Tops ...	3·36 %	2·85 %	4·14 %	4·56 %	- 15·2 %	+ 10·3 %
	Bottoms	3·47	3·60	3·89	3·60	+ 3·7	- 7·4
12 noon	Tops ...	4·52	0·08	4·26	0·40	- 98·0	- 90·4
	Bottoms	—	2·99	4·12	4·19	+ 9·14	+ 1·8
6 p.m.	Tops ...	4·14	0·64	3·92	0·61	- 84·6	- 84·5
	Bottoms	4·03	5·65	4·09	5·77	+ 40·3	+ 41·2
12 night	Tops ...	4·12	5·31	3·98	5·10	+ 29·1	+ 27·8
	Bottoms	—	—	4·15	3·18	—	- 23·3

laevulose follows more or less closely the general course of the curve of total hexoses (calculated as invert sugar, from reduction values only). But there is no real significance in this because with the dextrose values apparently constant, the laevulose figures, which are also calculated from the same reduction values, necessarily follow the figures for total hexoses (at any moment $D + L$ = total hexoses).

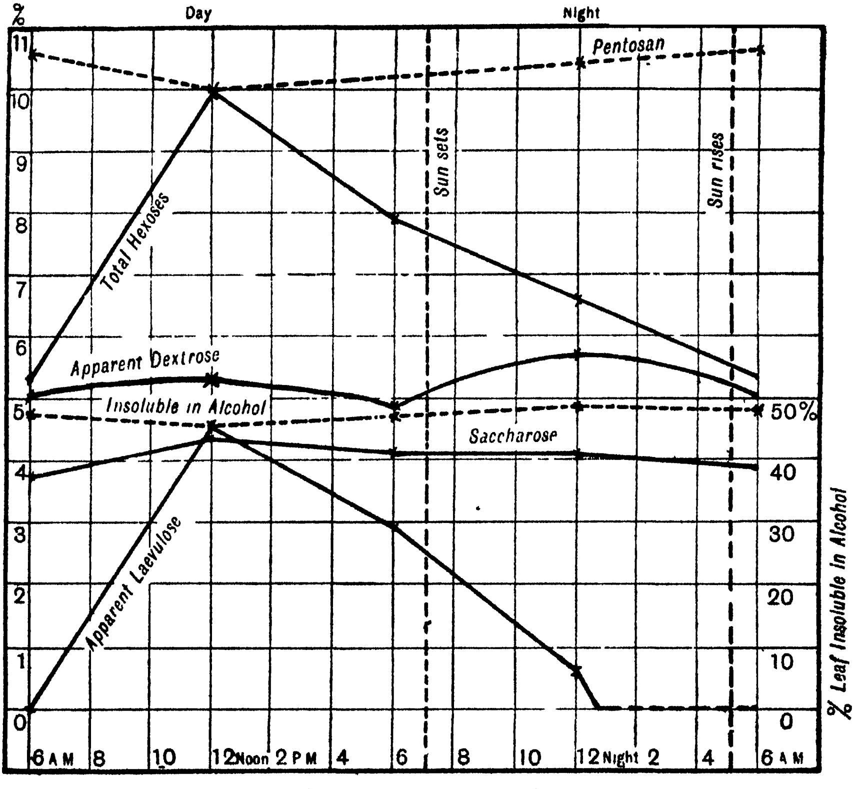

Fig. 4. Mangold stalks, tops, Series I, Aug. 26–27, 1913.

Exactly the same kind of relation can be traced between the fluctuation of the apparent hexoses and the values of Δ in the *bottom* halves of the stalks (Fig. 5). In this case, however, *both* the dextrose and laevulose appear to undergo wide variations. From 6 a.m. to noon the dextrose increases *absolutely* faster than the laevulose (from 8·55 to 11·0 per cent. for dextrose compared with a change from 0·2 to 1·8 per cent. for laevulose) although *relatively* the dextrose does not increase so rapidly as the laevulose, as shown by the fall of $\frac{D}{L}$ from 37·2 to 6·0.

The increase in the proportion of apparent dextrose is accompanied by the rise of Δ from a negative value (– 7·4, invertase figure, Table VIII) to a slightly positive value (+ 1·8). From noon to 6 p.m., although both dextrose and laevulose appear to be falling, the laevulose relatively falls faster than the dextrose $\left(\frac{D}{L}\text{ \textit{increases} from 6·0 to 9·1}\right)$ and the value of Δ increases to + 41 (Table VIII). Between 6 p.m. and midnight laevulose appears to fall little, but the dextrose rapidly, at the same

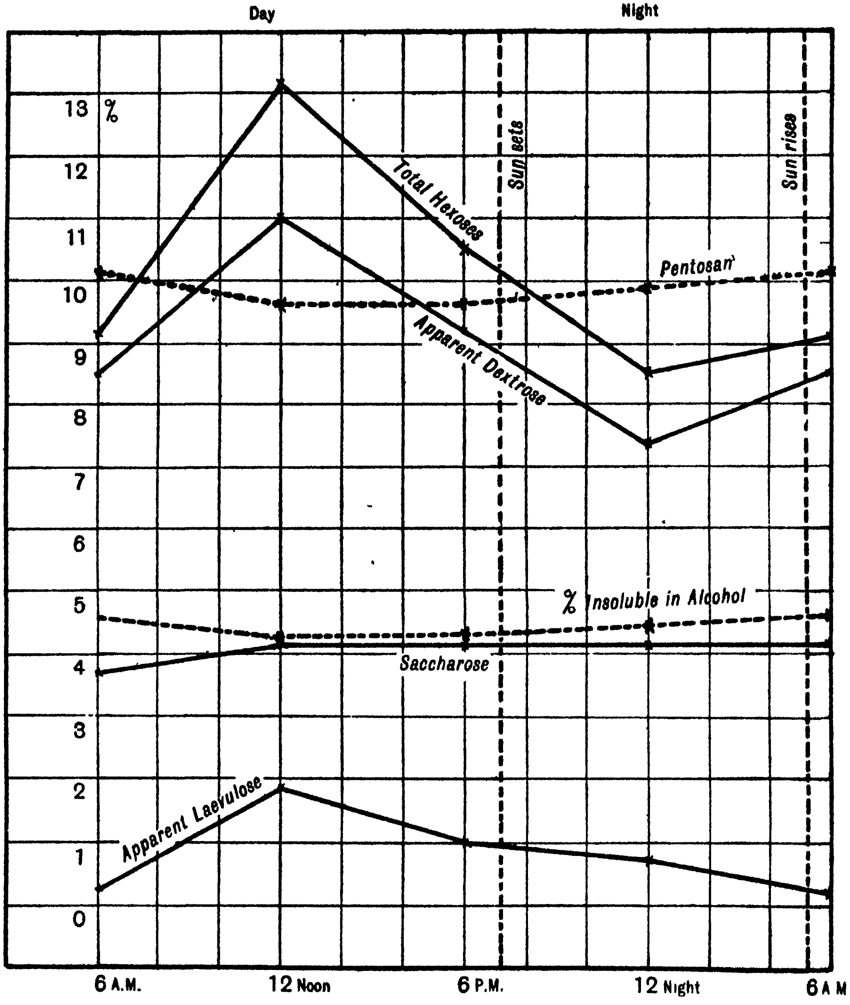

Fig. 5. Mangold stalks, bottoms, Series I, Aug. 26–27, 1913.

time Δ changes from + 41 per cent. to a negative value − 23 per cent. After midnight the apparent dextrose suddenly begins to rise again and the laevulose to fall; as would be expected the value for Δ becomes less negative (changes from − 23 to − 7·4).

As showing the gradual transference of the optically active impurities from the tops to the bottoms of the stalks, it is interesting to compare the values in Table VIII for say 6 a.m. with those for 12 noon. The impurity in the 6 a.m. tops is such as to cause Δ to have a positive value + 10·3 per cent. (invertase); at the same time, however, the bottoms have a negative value − 7·4, but at 12 noon the value of Δ for the bottoms has become positive, viz. + 1·8 (the sum of + 10·3 and − 7·4 is + 2·9). Similarly at 6 p.m. the value of Δ is negative in the tops (− 84·5) but positive in the bottoms, but at midnight the bottoms show a negative value, − 23·3; had the whole of the material causing the negative value at 6 p.m. been transferred to the bottoms, the change expected would be − 84·5 + 41·2 or − 43·3.

In the case of the stalk bottoms (Fig. 5), where the fluctuation of the apparent laevulose is relatively small, it is the dextrose curve which follows most closely the curve of total hexoses, but as pointed out in the case of the laevulose in Fig. 4 this has no real significance and is a result merely of the method of calculation.

If one compares merely the relative position of the apparent dextrose and laevulose curves in Figs. 4 and 5, dextrose seems to accumulate at the bottoms of the stalks far more than the laevulose, the values for dextrose (7·4 to 11·00 per cent.) being higher in Fig. 5 than in Fig. 4 (4·87 to 5·71), whilst the fluctuations of laevulose are smaller (0·23 to 1·8 per cent. in Fig. 5 as compared with 0 to 4·5 per cent. in Fig. 4). But from the considerations already brought forward it is clearly quite unsafe to conclude that it is actually dextrose which accumulates at the bottom of the stalks, as large quantities of other optically active substances are undoubtedly present, which cause the wide divergences between the results for cane sugar by the polarisation and reduction methods. If dextrose were the principal sugar present (in some cases it appears to be, as at 6 a.m., the sole hexose in the stalks) it would point, as assumed by Lindet, to the laevulose being largely consumed on the way from leaf to root for constructive purposes; but it would necessitate also that the saccharose in the root is built up from the dextrose being conveyed to it. This would involve a transformation in the root of dextrose into laevulose, followed by a synthesis of cane sugar from dextrose and laevulose. Whilst this operation is a possible one, it is more likely that the actual reducing sugars in the stalk reach the root as invert sugar[1] and that the apparent predominance of dextrose in the stalks is due solely to dextro-rotatory impurities; the existence of these is clearly proved by the enormous differences found in the cane sugar estimations by the polarisation method. Until really reliable methods of determining the true proportions of dextrose and laevulose have been devised it is impossible to draw any further conclusions on this point.

Series II. The considerations put forward above, correlating in Series I the apparent dextrose and laevulose values with the divergence between the cane sugar values determined by the reduction and polarisation methods, hold for the stalks and mid-ribs in Series II also[2]. The following table gives the values of Δ, and Figs. 9 and 10 of the preceding paper show the curves for dextrose and laevulose. It will be seen that, as in Series I, when the apparent laevulose increases rapidly as compared with dextrose the values of Δ become less positive; when the apparent laevulose decreases, the values become more and more negative.

Fig. 9 of the preceding paper shows the apparent variation of dextrose and laevulose in the stalks as compared with that of saccharose and the total hexoses and the variation of the ratio $\frac{D}{L}$. As in the tops of stalks in Series I the apparent dextrose rises slightly during the day (10 a.m. to 4 p.m.) but then remains practically constant until 4 a.m. next morning. The laevulose rises considerably more rapidly from 10 a.m.

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[1] It is quite possible that the ratio of dextrose to laevulose in the mixture of sugars reaching the root is not strictly 1, owing to one of the sugars being put more under contribution for purposes of growth or respiration in the leaves or stalks than the other. But it is probable that the ratio is very nearly unity as is the case in the leaves (September and October), when the amount of optically active impurities interfering with the determination is a minimum.

[2] The same principle can be applied to the leaves of Series II and III to explain the fluctuations of Δ, i e. the difference between the results found for cane sugar by double polarisation and by reduction, which are far less marked in the case of the leaves than with mid-ribs and stalks, because the proportion of optically active impurities is relatively less. In practically all cases when the apparent dextrose increases faster than the apparent laevulose, the divergence becomes increasingly positive; when the laevulose increases faster than dextrose the divergence becomes more negative. As pointed out on p. 339, when $\frac{D}{L}$ is unity there is the closest agreement between the results for cane sugar obtained by the two methods and the departure of the ratio $\frac{D}{L}$ from 1 is probably merely apparent and not real.

to 4 p.m. then falls until 11 p.m., when a second rise occurs. In the mid-ribs (Fig. 10 of preceding paper) the reverse is the case, the dextrose being nearly constant during the day and falling at night, whilst the laevulose falls by day and increases by night.

TABLE IX.

Divergence of Results for Saccharose in Stalks by Polarisation Method. Series II. September 10th–11th, 1912.

Time	% saccharose found				Polarisation results high by % (Δ)		$\frac{D}{L}$ (xylose)
	Citric inversion		Invertase inversion				
	Reduction	Polarisation	Reduction	Polarisation	Citric inversion	Invertase inversion	
10 a.m.	5·25	5·82	4·39	—	+ 10·7	—	3·33
4 p.m	5·75	4·70	4·78	6·59	− 17·9	+ 35·0	2·34
11 p.m.	5·18	8·26	—	6·67	+ 60·0	—	4·56
4 a.m.	5·34	5·38	5·10	6·45	+ 0·8	+ 26·5	3·30
6 a.m.	5·25	5·68	4·88	4·82	+ 8·4	+ 1·9	2·84

SUMMARY.

1. It is shown that in the extracts of mangold leaves and stalks optically active impurities are always present which are not precipitated by basic lead acetate and hence vitiate the estimation of the dextrose and laevulose. These substances are possibly acid amides (such as glutamine and asparagine) or amino-acids (such as glutamic and aspartic acids) which form soluble lead salts.

2. These impurities occur in the leaves, but are much more abundant in the mid-ribs and stalks.

3. In the leaves the dextrose and laevulose appear to be present in approximately equal amount, as would be expected if they were formed from saccharose by inversion. When the ratio $\frac{D}{L}$ departs from unity it is probably owing to the presence of a dextro-rotatory impurity (glutamine?) which increases the amount of dextrose apparently present; but at certain times of the day a laevo-rotatory impurity seems to predominate so that the ratio $\frac{D}{L}$ becomes less than unity.

4. In the mid-ribs and stalks, especially at the bottoms of the latter, the dextrose always appears to be in very large excess as compared with the laevulose; this is probably due to the proportion of the dextrorotatory impurity being relatively greater in these parts than in the leaf, as is shown by the divergences between the polarisation and reduction values of saccharose being far greater.

5. The apparent fluctuations in the *ratio* of dextrose to laevulose are probably due to fluctuations in the optically active impurities rather than to variations in the sugars themselves. This is shown by the fact that these fluctuations can be correlated with the differences between the cane sugar values as determined by reduction and polarisation. When the apparent laevulose increases faster than the dextrose the results for cane sugar obtained by polarisation are *lower* than the reduction values; when the apparent dextrose increases faster than the laevulose or the laevulose falls more rapidly than the dextrose, the polarisation results are in excess of the true values.

6. The fluctuations of the apparent dextrose and apparent laevulose take place more or less regularly during the 24 hours; this points to a regular variation in the optically active impurities.

7. In the *leaves* the values of saccharose obtained by the double polarisation method are almost always *higher* than the reduction values; in the stalks, however, they are sometimes very high and sometimes very low. This is probably due to the presence of at least two different optically active substances at different times of the day. The increase of the apparent laevulose corresponds with the increase of the substance causing low results for cane sugar by the double polarisation method; the increase of apparent dextrose corresponds with a falling off of this substance and the formation of the impurity which gives high results.

8. Until more reliable results can be obtained for the true dextrose and laevulose by methods which are independent of the polarimetric data, it seems justifiable, from the results brought forward, to assume that the dextrose and laevulose exist in the leaves and stalks as invert sugar and travel in nearly, if not exactly, equal proportions to the root, where retransformation into saccharose occurs. This assumption best agrees with the regular rise and fall of the total hexoses in the stalks and mid-ribs along almost straight lines during the night, as contrasted with the more irregular fluctuation of the apparent dextrose and laevulose taken separately.

9. It is impossible in the present state of our knowledge to draw any conclusions from the proportion of apparent dextrose or laevulose

in plant tissues as to whether either of these sugars is better adapted than the other to tissue formation or to respiration. All such conclusions in the past are valueless because the analytical methods at present existing do not give true values for these sugars.

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(*Received October* 4, 1915.)

STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS.

III. THE CARBOHYDRATES OF THE LEAF AND LEAF STALKS OF THE POTATO. THE MECHANISM OF THE DEGRADATION OF STARCH IN THE LEAF.

By WILLIAM A. DAVIS and GEORGE CONWORTH SAWYER[1].

(Rothamsted Experimental Station.)

In the first two papers of this series (pp. 255–351) we have dealt with the carbohydrates of the mangold, a plant which, like its near relation the sugar beet, stores only saccharose in its root. One of the most striking features of this plant is that it forms no starch in the leaf, except during the very earliest stages of growth when it is a seedling; it is only during this period, when the root is very small and has not developed sufficiently to store the sugars formed, that starch appears in the leaf at all. When the mangold has begun to develop a large storage reservoir in the root and the sugar can be readily translocated away so that all danger is avoided of too high a concentration in the leaf, starch ceases to be produced, and during the whole of the growth in August, September, and until the roots are lifted at the end of October, it is entirely absent from the leaf. Maltose too is entirely absent. In these respects the mangold, although a dicotyledon, resembles monocotyledonous plants such as the onion (*Allium cepa*) and snowdrop (*Galanthus nivalis*), which do not form starch in the leaf although they store both starch and inulin in the bulb; in many respects, as we have shown, the phenomena of formation and translocation of sugars in the mangold are similar to those observed by Parkin [1912] in the snowdrop. It appears, however, that in the later stages of growth (September and October) certain gummy substances, which were not studied in any detail, are formed as a reserve in the leaf tissue and appear to be broken down to sugars at night, thus playing a similar part to the starch in most foliage leaves.

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[1] Mr A. J. Daish, who shared our earlier work, would have taken part in this investigation had not his military duties, after the outbreak of war, rendered it impossible. He assisted us during the heavy work of the 24 hours picking of July 16–17th, 1914, and we wish here duly to acknowledge this.

In view of the fact that we found, by using our method of estimating maltose by maltase-free yeasts, that maltose is entirely absent not only from the leaf and stalks of the mangold, which does not store starch, but also from the leaves of many other plants which form an abundance of starch, we considered it desirable, in order to test Brown and Morris' views [1893] as to the part played by diastase during the night in breaking down the starch to maltose, to study the variation of the carbohydrates in the potato leaf throughout a complete 24 hours period. The potato forms considerable quantities of starch in its leaf and if, as seemed possible, maltose is an intermediate stage in the synthesis of starch, just as it is in its degradation by enzymes, it should appear in the leaf, at least in small quantities, during the day; if the starch is broken down by ordinary diastase in the way suggested by Brown and Morris, maltose should appear in increasing quantities at night during the disappearance of the starch from the leaf. Finally, if Brown and Morris' view [1893, p. 673] is correct that maltose is the translocation form of starch, maltose should be found in the stalks.

Our experiments (see Tables I and II, p. 366) show that maltose is entirely absent from the leaf and stalks of the potato at all periods of the day and night. We have now made nearly 500 analyses by means of maltase-free yeasts of many different kinds of plants, including the nasturtium (*Tropæolum majus*), turnip, carrot, sunflower (*Helianthus annuus*), dahlia, *Arum maculatum* and vine (*Vitis vinifera*); *in no case has maltose been found either in the leaf or stalks*, even in such plants as the turnip or nasturtium which store very large quantities of starch in the leaf[1]. We need here only refer to the data given in a previous paper (Davis and Sawyer [1914]) for the quantitative fermentations carried out with the alcoholic extract of the turnip leaf (starch = 12·77 per cent. of the total vacuum-dried leaf). In order to work with as large a quantity of substance as possible 1 litre of the purified aqueous solution of the sugars (a quantity which represented 44·69 grms. of the total vacuum-dried leaf matter) was evaporated *in vacuo* to 175 cc. and made up to 250 cc. Three portions of 50 cc. each were fermented during 3 weeks to 1 month with *S. marxianus* and *S. exiguus*, and after treatment with alumina cream made up to 100 cc. Two duplicate fermentations were carried out with a pure culture of distillery yeast.

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[1] In one case (July 9th, 1913) the leaf of the turnip was found to contain 18·73 per cent. of starch calculated on the vacuum-dried matter left after extracting the sugars, etc., with alcohol; this calculated on the total vacuum-dried matter of the leaf, including the sugars, becomes 12·79 per cent. In a sample of *Tropæolum* leaf (July 4th, 1913) the starch formed 26·75 per cent. of the dry leaf after extraction, or 17·6 per cent. of the total vacuum-dried matter of the original leaf.

50 cc. of the filtrate (representing 4·469 grms. of T.V.D.M.) gave in the case of the *S. marxianus* and *S. exiguus*, 0·0524 grm. CuO; and gave in the case of the distillery yeast, 0·0500 grm. CuO.

These values are practically identical and well within the range of error of the method. They show that *maltose was entirely absent from the turnip leaf* in question. The reducing power, as pointed out [1914], is to be attributed to unfermentable *pentoses*; it corresponds with 0·51 per cent. of pentose; 0·60 per cent. was found by the ordinary phloroglucinol method when applied directly to the solution containing the sugars, prior to fermentation.

Brown and Morris, in their important memoir, gave what was undoubtedly good evidence of the presence of maltose in their extracts of *Tropæolum* leaf. They were not content merely with the analytical data but endeavoured "in view of the immense importance which must necessarily be attached to this product of starch hydrolysis to obtain more direct evidence of its presence." They succeeded in isolating an osazone, apparently identical with maltosazone, from the solution of the mixed sugars contained in a large quantity of the dry leaves of *Tropæolum*, and analysed it. Finally they showed the presence of maltose by treating a solution of the mixed sugars of the leaf, after completely inverting the saccharose, with a preparation of the enzyme maltase. This enzyme always brought about a large increase in the cupric reducing power, amounting generally to about 75 per cent. of the increase observed on digesting the same solution with dilute acid.

It is impossible in view of these facts to doubt that maltose was present in the material worked with by Brown and Morris. It is, however, possible to reconcile these results with our own by taking into account the difference between the methods of extraction of the sugars adopted by Brown and Morris and by ourselves. We have been led to conclude that plant leaves which store starch contain in addition to the enzymes of ordinary diastase (a fact which was first definitely proved by Brown and Morris) the enzyme *maltase*, which is capable of breaking down maltose to dextrose. We have shown in a previous paper

(Davis and Daish [1914]) that taka-diastase, the mixture of enzymes isolated from *Aspergillus oryzae*, differs from the ordinary diastase of malt extract mainly in containing maltase in addition to the ordinary starch resolving enzymes. Taka-diastase therefore converts starch paste completely into a mixture of maltose and dextrose, the latter rapidly increasing in amount until 80–85 per cent. of the sugar is in this form. We consider that the ordinary foliage leaf contains a mixture of enzymes similar to that elaborated by *Aspergillus oryzae*, and of such a nature that the maltase is always present in relative excess so that the maltose formed by the breaking down of the starch is very rapidly and completely converted into dextrose. Now in our method of preparing the leaf samples for analysis, the material was dropped into boiling alcohol containing a little ammonia, so that the enzymes were destroyed instantly, but Brown and Morris and most other workers in this field *dried their leaf material in an oven before extracting the sugars.* During this drying, owing to the large quantity of moisture in the leaf, the temperature only rises gradually and the enzymes continue to act for a considerable time before they are destroyed. Maltase is the first of the enzymes to be put out of action; it is well known to be one of the most unstable of enzymes. Our experiments (Davis, 1914, I) with taka-diastase show that it is largely destroyed before a temperature of 55° is reached. When leaves are dried in an oven, after the maltase has been destroyed at say 50° C., the ordinary diastatic enzymes continue to act under optimum conditions as regards temperature and considerable quantities of starch are broken down to dextrin and maltose. This action lasts until the temperature rises to about 80°, when the dextrin and maltose-forming enzymes are also destroyed. As the maltase has been completely killed in the earlier period of drying, the maltose formed in this way will persist as such and be found in the mixture of sugars subsequently extracted from the dried material.

In support of this explanation of the differences between Brown and Morris' results and our own, several facts may be adduced. In Brown and Morris' experiments the proportion of starch in the freshly plucked nasturtium leaf at the end of a sunny day was found to range between 2·9 and 7·4 per cent. of the total dry matter; we have always found the starch in the same leaf to be considerably higher, thus in the example given on p. 353, footnote, the starch was 17·6 per cent. of the total vacuum-dried matter or about two and a half times the highest figure given by Brown and Morris. In one case cited by these workers the starch was found to be 4·59 and the maltose 5·33 per cent., the sum of the two being

9·9 per cent.; this is still considerably lower than the values we have found. Kluyver [1914] recently on repeating Brown and Morris' experiments with *Tropæolum*, but using a new biochemical method of estimating the hexoses, saccharose and maltose[1], based on the differences in the amount of carbon dioxide evolved on fermenting the solution with special *torulae* and with ordinary yeast, also found in all cases relatively very small amounts of maltose. Thus in one case, which is of special interest because the hexoses and cane sugar are nearly identical with values found by Brown and Morris, Kluyver found in leaves plucked at 2.30 in the afternoon, hexoses = 5·2 per cent., saccharose = 4·6 per cent., *maltose* = 0·3 per cent.; this compares with Brown and Morris' analysis, hexoses = 5·6 per cent., saccharose = 4·9 per cent., *maltose* = 1·2 per cent. In the remaining cases which Kluyver cites the results are given merely relatively, without calculating back on the dry matter of the leaf: but, in every instance, the proportion of maltose found was exceedingly small as compared with the saccharose and hexoses. Thus, for example, a sample of *Tropæolum* leaf picked at 4 p.m. on July 28th, and therefore corresponding with an analysis in which Brown and Morris found saccharose 8·02 per cent., maltose 3·62 per cent., and in which maltose formed 27·5 per cent. of the total sugars, gave

Saccharose	...	25·8 mgrms.
Hexoses	...	21·8 ,,
Maltose	...	1·6 ,,

The maltose found here forms only 3·2 per cent. of the total sugars. In Brown and Morris' experiments the maltose always appeared to be at a maximum at the end of the afternoon, that is at the same time as the starch reached its highest values; for example, in one case (p. 669) a leaf picked at 5 p.m. when the starch formed 4·59 per cent. of the dry leaf, maltose was found to be present to the extent of 5·33 per cent., *and to form* 56 *per cent. of the total sugars.*

These results are fundamentally different from Kluyver's obtained at the same time of day and the difference is probably to be explained by the fact that in Brown and Morris' experiments the heating up of the leaves in drying was much slower and allowed far more diastatic action to occur than in Kluyver's experiments. Kluyver especially points out that his leaves, which were dried in thin layers in a baker's oven heated to 105°, were exposed to the drying process during only 5 to 10 minutes. We have ourselves made several experiments with *Tropæolum* leaves dried *rapidly* in a steam oven and by our own methods have invariably found *no* maltose to be present, just as in the case of the same leaves dropped into boiling alcohol.

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[1] *Torula monosa* does not contain the enzymes maltase and invertase, and hence is capable of fermenting the hexoses only, leaving the maltose and saccharose unchanged; *Torula dattila* contains invertase but not maltase, and therefore ferments cane sugar and the hexoses but not maltose. Dr A. J. Kluyver has been kind enough to send us pure cultures of these Torulae, which Dr H. Limbosch has tested for us in our laboratory, according to our own methods of working, on very carefully purified specimens of sugars. We can confirm Kluyver's statements as to the specific nature of these organisms which should prove of considerable service in sugar analysis of the kind we have had to deal with.

From the above facts we have concluded that the maltose which was undoubtedly present in Brown and Morris' experiments in relatively large amounts and in Kluyver's experiments in far smaller proportions owing to the greater rapidity of drying, was not formed in the tissue of the leaf as such during growth, but was produced by the degradation of starch by the diastatic enzymes remaining after the maltase in the leaf had been destroyed in the first stage of the drying process. As regards the mechanism by which starch is utilised in the plant when, at the end of the day, the reserves in the leaf are called upon, *we consider that the starch is hydrolysed completely to dextrose by the leaf enzymes*, which resemble the enzymes of *Aspergillus oryzae* in containing an abundance of maltase. Brown and Morris' main view that the starch is utilised by a purely enzymic process seems to us perfectly correct, but we regard the enzymic degradation as stopping, not at maltose, as supposed by Brown and Morris, but at the stage of dextrose, the final product of starch hydrolysis. One of us has shown (Davis, *Chemical World*, 1914, p. 271) that yeasts which do not contain the enzyme maltase, for example, *S. anomalus* and *S. exiguus*, are quite unable, even when in the throes of starvation, to make use of maltose in the solution in which they are growing; similarly we find that *Torula monosa*, which does not contain invertase, is unable to make use even of cane sugar. Plant tissue, we consider, in exactly the same way before it can utilise starch, maltose or saccharose, for purposes of growth, must break these substances down to the simple hexoses by enzyme action. This view explains the significance of the fact that the sugars in the stalks of all the plants we have examined consist largely of the simple hexoses; these sugars are capable of being directly assimilated by the cambium layer of the stems or by other growing points. The necessity of transformation of saccharose into invert sugar thus explains the almost ubiquitous presence of invertase in the plant, except in such storage reservoirs as the mangold root, where cane sugar is permanently housed.

The views we put forward are in accord with modern views, based largely on the work of Abderhalden and his school, as to food assimilation by animals; in all cases it is necessary for such food, for example, proteins, to be broken down by enzymes into its simplest components or "*Bausteine*," which are then taken up by the different cells or tissues and synthesised afresh.

The theory we have given of the method by which starch is broken down in the leaf would lack justification unless definite evidence of the presence of maltase in leaf tissue could be brought forward. At the suggestion of one of us, Mr A. J. Daish has made a special study of this question. In a series of experiments, details of which will be published later, he has found that maltase is always present in the leaf tissue he has examined when starch is also present. Little doubt therefore can be entertained of the correctness of the view we put forward that starch is broken down in the leaf to dextrose. The fact that maltose can never be detected either in the leaf or stalks of plants points to the amount of maltase always being in relative excess in the cells where the starch degradation actually occurs, so that it is able to deal instantly with the whole of the maltose formed from the starch. The fact that maltose, unlike cane sugar, never occurs in the stalks or conducting vessels is probably due to the fact that maltase is an intracellular enzyme and apparently acts in close collaboration and in the immediate proximity of the ordinary diastase which first attacks the starch in the cells where this substance is stored.

Cane sugar is apparently the first sugar formed in the potato leaf and is transformed into hexoses for translocation.

The most striking point which appears when the analyses of the potato leaves and potato stalks are compared (see Tables I and II) is that whereas *the saccharose is greatly in excess of the hexoses in the leaf, the reverse is true in the stalks.* These results are exactly similar to those obtained with the mangold leaf in the early stages of growth (Series I), a fact which points to the mechanism of formation and translocation being the same in both cases. Saccharose is probably the first sugar formed in the mesophyll of the leaf; it is gradually inverted on its way through the veins, mid-ribs, and stalks, the inversion becoming more and more complete as the root or tuber is approached. In this series of pickings it must be borne in mind that the "stalks" were mainly those bearing the small leaflets and did not include any of the stem in the neighbourhood of the tuber where, by analogy with the mangold and snowdrop (Parkin [1912]), the hexoses would be found probably to preponderate even more than is shown in Table II. Time has allowed us only to take one series of pickings with the potato, but it seems highly probable that, as in the case of the mangold, sugar beet, and snowdrop, the proportion of hexoses to saccharose becomes greater and greater in both leaf and stalk as the season advances, and the storage function becomes more and more predominant.

As regards the transformation of the hexoses into starch in the tuber, it is interesting to note that in this way the hexoses are as it were imprisoned and held until required for later use, when the appropriate enzymes again degrade the starch to sugars. In the mangold the imprisonment of the hexoses in the root is effected by their transformation into cane sugar.

From data which we have obtained with many other plants, to be published later, it appears that cane sugar is produced, generally in a predominant proportion, in the leaf of *all* plants, whatever be the form in which the sugars are finally stored (cane sugar, starch, inulin or dextrose). Thus, for example, we find that, when proper precautions are taken to prevent enzymic change, contrary to Deleano's [1912] recent statement, cane sugar is the principal sugar of the vine leaf (*Vitis vinifera*). In this plant the storage form is dextrose, and unless the cane sugar is a primary product of the mesophyll tissue it is difficult to see any special reason for its predominance in the leaf. If dextrose and dextrose alone were, according to Strakosch's [1907] views, the direct product of photosynthesis, one would expect to find it the principal if not the sole sugar in the leaf of a plant which stores dextrose as its reserve carbohydrate. In fact, as stated in our previous paper (I), all the data we have obtained with plants of many different kinds best harmonise with the view put forward by Brown and Morris [1893], that saccharose is the first sugar formed in photosynthesis and that the hexoses are formed from it and not *vice versa*. It seems to be the general *function of the mesophyll tissue to elaborate saccharose directly*; this is broken down in the veins, mid-ribs and stalks, and reaches the place of storage in the form of hexoses. Unless saccharose is a primary product it is difficult to see why it should predominate in the leaves of plants of such different types as the potato, the vine, sunflower and snowdrop, in none of which is cane sugar the storage form; there seems, indeed, no useful purpose in its production at all in such cases, as the substances stored are undoubtedly built up from hexoses, which are the predominating constituents of the sap in the *stalks*, and could very well be translocated directly as such from the leaf. It is possible, and may be argued, that the saccharose in the leaf serves to regulate the osmotic pressure, owing to the ready interconversion of saccharose and hexoses; but in plants which form starch, such as the potato, this regulation could be quite as well effected by the precipitation of the polysaccharide and the function of the cane sugar is not easily understood unless it be regarded as a primary and compulsory product of the mesophyll.

The Dextrose-Laevulose Ratio. As was the case in the mangold leaf, it is shown that it is impossible to obtain accurate values for dextrose and laevulose owing to the presence in the solutions of optically active impurities which are not removed by the ordinary process of defecation by basic lead acetate. These impurities also interfere with the estimation of the saccharose by the double polarisation method and, as in the mangold, the fluctuations of the apparent dextrose and laevulose can be correlated with the divergences between the values found for saccharose by the reduction and by the optical methods (see p. 344). It appears that *two* optically active impurities with rotations of opposite sign are formed at different periods of the 24 hours, and it is the variation of these that causes the apparent fluctuations in the proportion of dextrose and laevulose. In the leaf a substance with a laevo-rotatory power generally predominates, so that the laevulose appears to be greatly in excess of the dextrose; but in the stalks this is no longer the case and dextrose appears to be largely in excess of the laevulose.

EXPERIMENTAL.

The methods of sampling, extraction, and analysis were the same as those described in the case of the mangold (see Paper I). The potatoes (King Edward VII) were grown on a piece of ground at the side of the laboratory; at the date of picking (July 16th–17th, 1914) the plants were just beginning to form flower buds and the tubers were small. Rain had fallen heavily on July 12th, but the days following were dry and sunny. Pickings were taken every two hours. The leaflets were detached from the rachis, but the mid-ribs of these leaflets were not cut out so that the results given for "leaves" refer to the whole leaflets including these mid-ribs; what we have called "stalk" consisted in reality mainly of the rachis of the compound leaves and included only a small portion of the main stalk or stem, namely the portion furthest from the tubers.

Estimation of Starch and "Soluble Starch."

The dried potato leaf obtained after completely extracting the sugars and other substances soluble in 80 per cent. alcohol was found to contain large quantities of a substance readily soluble in water and possessing a high positive rotation. This made it necessary to modify the method of estimating starch which we have employed (Davis and Daish [1914]) by first completely extracting this substance with water from the portion of material used in the analysis. At certain times of the day (4 p.m. to 8 p.m.) the aqueous extract so obtained contained a substance which resembled soluble starch or dextrin in yielding a mixture of maltose and dextrose on treatment with taka-diastase. In all cases the reducing power (if any) and rotatory power of the aqueous extract were determined after diluting to a known volume (250 cc.); an aliquot portion (150 cc.) was then treated with taka-diastase, and, after the conversion, with basic lead acetate (which generally produced a copious precipitate owing to the presence of tannins, etc.), being then diluted to a known volume (200 cc.). The reducing and rotatory powers of the solution were determined and from the change in these brought about by the taka-diastase the "soluble starch" (or dextrin) was calculated. In most cases the "soluble starch" was *nil*, but between 4 p.m. and 8 p.m. considerable quantities could be detected. Even in these cases, however, the amount of soluble starch found in this way did not account for more than 25 to 50 per cent. of the rotation observed in the aqueous extract; in all cases, too, the basic lead acetate added after the conversion produced a heavy, gelatinous precipitate, pointing to the presence of tannins, gums, etc. The aqueous extract before conversion invariably had a slight cupric reducing power (50 cc. of the 250 cc. gave 0·01 to 0·02 grm. CuO) which may perhaps have been due to unextracted sugars; but as in the experiments with mangold leaves, the extraction of sugars was always complete, it is probable that the reduction was due to a substance of the tannin class. For purposes of comparison we give in Table I the actual values calculated for the rotation $(\alpha)_D$ in a 200 mm. tube of the aqueous extract of the leaf material corresponding with 100 grms. of the *total vacuum-dried matter* of the leaf (including the sugars and alcohol soluble substances). The value is also given for the "soluble starch" ($[\alpha]_D = 202°$) that this would correspond with, calculated as a percentage on the total vacuum-dried matter. Thus a comparison can be made of the true soluble starch found by the taka-diastase and the value found in this way from the rotation.

The true *starch* in the leaf was estimated by treating with taka-diastase the leaf material remaining after extraction with water; this was, of course, first gelatinised by boiling water in the usual way.

The following is an actual example showing the method of analysis and calculation.

Potato Leaf, 8 p.m., July 16th, 1914.

10·7148 grms. of the dried powdered leaf material remaining after the extraction of sugars[1], dried at 110° *in vacuo* until the weight was constant gave 9·8405 grms.; the moisture present therefore = **8·16** per cent.

The vacuum-dried weight of matter soluble in alcohol for this picking was 59·25 grms.

The weight of oven-dried matter (fibre, starch), etc., left after extraction with alcohol was 111·55 grms.; as the moisture in this was 8·16 per cent., the vacuum-dried weight = 102·42 grms.

The total vacuum-dried matter (T.V.D.M.) therefore

$$= 102{\cdot}42 + 59{\cdot}25 \text{ grms.} = \mathbf{161{\cdot}67} \text{ grms.}$$

The 9·8405 grms. of leaf substance was transferred to a beaker flask of 250 cc. capacity and left with about 200 cc. of water and 2 cc. of toluene for 24 hours at 38°, stirring well at intervals. The clear solution was then decanted through a starch-free filter paper as completely as possible and the residue washed by decantation until the volume in the flask was 250 cc.

Aqueous extract. 50 cc. of the 250 cc. gave 0·0217 grm. CuO.

Polarisation in 200 mm. tube = + 0·358° (sodium flame, 20°). This rotation calculated as 100 grms. of vacuum-dried extracted leaf

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 100}{9{\cdot}8405} = 9{\cdot}10^\circ.$$

Calculated on 100 grms. of T.V.D.M. $= \dfrac{9{\cdot}10 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{5{\cdot}76}^\circ.$

Calculated as soluble starch ($[\alpha]_D = 202^\circ$) per 100 grms. of T.V.D.M.

$$= \frac{0{\cdot}358 \times 2{\cdot}5 \times 10^4 \times 102{\cdot}42}{2 \times 202 \times 9{\cdot}8405 \times 161{\cdot}67} = \mathbf{1{\cdot}43} \text{ per cent.}$$

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[1] This had been dried in an oven at 100°, ground in a mill and kept in a stoppered bottle until the analysis was made. For precautions in sampling this material see Davis and Daish [1914], p. 161.

"*Soluble Starch*" (*or dextrin*) *in Aqueous Extract.*

150 cc. of the 250 cc. were left with 0·1 grm. of taka-diastase and 1 cc. of toluene for 24 hours at 38°; to the solution 5 cc. of basic lead acetate solution were then added, which was *just* sufficient to precipitate the whole of the tannins, gums, etc. The solution was diluted to 200 cc. at 15° and filtered; the slight excess of lead in the filtrate was *exactly* precipitated by adding solid sodium carbonate and the reducing and rotatory powers of the filtrate determined.

50 cc. of the 200 cc. gave 0·0718 grm. CuO.
Rotation in 400 mm. tube at 20° = + 0·202°.

Correcting for 0·1 grm. taka-diastase, under exactly similar conditions (correction for CuO = 0·0360 grm.; for polarisation = + 0·106°), we have

CuO due to sugars present = + 0·0358 grm.
Polarisation due to sugars present = + 0·096°.

It is necessary to correct for the reducing power and polarisation of the original solution; for the reducing power we have

$$\frac{150}{200} \times 0{\cdot}0217 = 0{\cdot}0163 \text{ grm.}$$

As to the rotatory power, measurements made with the various pickings in which "soluble starch" was entirely absent showed that if the reducing substances be assumed to be sugars, with a cupric reducing power 2·5 grms. CuO per grm., they had the specific rotatory power $[\alpha]_D^{20°} = +25°$. The assumption that this is the case when the soluble starch is present will give no sensible error; we have therefore α_D due to these substances in a 400 mm. tube

$$= \frac{0{\cdot}0163}{2{\cdot}5} \times \frac{25 \times 400 \times 2}{10^4} = +0{\cdot}013°.$$

We have therefore as final values for maltose and dextrose formed by the diastase conversion:

CuO ex 50 cc. = 0·0358 − 0·0163 = 0·0195 grm.
Polarisation in 400 mm. tube = 0·096 − 0·013 = 0·083°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0195$$
$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}0830$$

Solving, $x = 0{\cdot}00443$ grm.; $y = 0{\cdot}00586$ grm.

The total dextrose corresponding with leaf material taken

$$= 0{\cdot}00443 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0296 \text{ grm.}$$

The total maltose corresponding with leaf material taken

$$= 0{\cdot}00586 \times \frac{200}{50} \times \frac{250}{150} = 0{\cdot}0391 \text{ grm.}$$

"Soluble starch" corresponding with dextrose

$$= 0{\cdot}0296 \times 0{\cdot}9 \quad = 0{\cdot}02664 \text{ grm.}$$

"Soluble starch" corresponding with maltose

$$= 0{\cdot}0391 \div 1{\cdot}055 = 0{\cdot}0371 \text{ ,,}$$

$$\text{Total} \quad = 0{\cdot}0637 \text{ ,,}$$

$$\text{Percentage of "soluble starch"} = \frac{0{\cdot}0637}{9{\cdot}8405} \times 100 = 0{\cdot}65.$$

∴ Percentage of soluble starch on T.V.D.M. in leaf

$$= \frac{0{\cdot}65 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{0{\cdot}41}.$$

True starch. The leaf material remaining after the extraction with water was gelatinised by heating with about 200 cc. of water during 30 minutes in boiling water; after cooling, 0·1 grm. taka-diastase was added and 2 cc. of toluene and the mixture left 24 hours at 38°, stirring at intervals. Two drops of concentrated sodium hydroxide were then added to destroy the enzyme and the solution filtered from the leaf material on a Buchner funnel; this was thoroughly washed with water by decantation until the total volume of the filtrate was about 475 cc., Basic lead acetate was then added (2·5 cc. was generally just sufficient) and the volume made up to 500 cc. The slight excess of lead in the filtrate was removed by adding *exactly* the necessary quantity of solid sodium carbonate and after filtering the reducing power and rotation of the solution were determined.

50 cc. gave 0·0908 grm. CuO

Polarisation = 0·264° in 400 mm. tube at 20°.

These values corrected for 0·1 grm. of taka-diastase under exactly similar conditions (CuO correction = 0·0138 grm.; polarisation = + 0·073°) give

Corrected CuO from 50 cc. = 0·0770 grm.,

Corrected polarisation = + 0·191°.

If x = dextrose in 50 cc.; y = maltose in 50 cc.,

$$2{\cdot}58x + 1{\cdot}38y = 0{\cdot}0770$$
$$4{\cdot}22x + 11{\cdot}01y = 0{\cdot}191^\circ.$$

Solving, $x = 0{\cdot}02587$ grm. in 50 cc.

$y = 0{\cdot}00746$ grm. in 50 cc.

Total dextrose in 500 cc. = 0·2587 grm. = 0·2328 grm. starch.

Total maltose in 500 cc. = 0·0746 grm. = 0·0707 „ „

∴ Total starch = 0·3035 grm.

Percentage of starch in the vacuum-dried extracted leaf

$$= \frac{0{\cdot}3035 \times 100}{9{\cdot}8405} = 3{\cdot}08.$$

Percentage of starch in the total vacuum-dried matter

$$= \frac{3{\cdot}08 \times 102{\cdot}42}{161{\cdot}67} = \mathbf{1{\cdot}95}.$$

SUMMARY OF ANALYSIS[1].

Rotation of aqueous extract calculated on 100 grms. T.V.D.M. in 100 cc.	= 5·76°
This represents as "soluble starch"	= 1·43 %
Actual "soluble starch" found by diastase	= 0·41 %
True starch found by diastase	= 1·95 %

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[1] A duplicate analysis of this sample gave: Rotation of aqueous extract per 100 grms. T.V.D.M. = 5·58°; calculated as soluble starch this = 1·38 per cent. "Soluble starch" by diastase = 0·30 per cent.; true starch = 1·24 per cent. The duplicates here given for the true starch are not so close as is usually the case in these analyses; a more typical case (4 a.m.) gave 1·24 and 1·43 per cent. The greatest difficulty is encountered in the sampling, as was pointed out in a former paper; for each analysis the whole of the powdered leaf material, especially that at the bottom of the bottle, where the heavy starch grains tend to collect, should be turned out on a sheet of paper and 10 grms. sampled so as to represent a fair average of the whole of the material.

RESULTS OF ANALYSES.

TABLE I.

Potato Leaves, July 16th–17th, 1914.

July 16th. Sun rises 4.2 a.m. July 17th. Sun rises 4.4 a.m.
Sun sets 8.10 p.m.

Time	Temp. °F.	% T.V.D.M. soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S./C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	Remarks
			Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M.	α_D calc. as sol starch %			
6 a.m.	57	37·2	2·16	2·11	+0·05	2·14	0·40	0·19	0·35	5·72	0·00	6·02°	1·49	0·00	1·88	Sunny
8 a.m.	60	39·1	2·47	2·60	−0·13	2·53	1·00	0·39	0·37	5·37	,,	8·32	2·06	,,	2·00	Sunny
10 a.m.	61	38·5	2·81	2·65	+0·16	2·73	0·37	0·14	0·52	5·30	,,	3·36	0·83	,,	2·55	Slightly overcast
12 noon	62	39·3	3·39	3·19	+0·20	3·29	1·21	0·37	0·43	5·35	,,	5·06	1·24	,,	1·40	Shower 1.30
2 p.m.	63	34·1	3·81	3·50	+0·31	3·66	0·67	0·18	0·44	5·40	,,	5·30	1·31	,,	1·81	Shower 3.30
4 p.m.	63	36·2	3·56	3·34	+0·22	3·45	0·93	0·27	0·42	5·33	,,	8·88	2·20	0·58	1·56	Very bright
6 p.m.	64	35·8	3·46	3·22	+0·24	3·34	1·27	0·38	0·46	5·42	,,	8·96	2·22	1·00	5·95	Bright
8 p.m.	60	36·6	2·77	2·69	+0·08	2·73	1·22	0·45	0·42	5·51	,,	5·67	1·40	0·36	1·61	
																Dark 9.15 p.m.
10 p.m.	59	34·8	2·76	2·49	+0·27	2·63	0·40	0·15	0·45	5·35	0·00	5·02	1·24	0·00	2·60	
12 night	56	36·6	2·48	2·30	+0·18	2·39	0·73	0·30	0·44	5·60	,,	8·32	2·05	,,	0·24	
2 a.m.	55	37·5	2·38	2·26	+0·12	2·32	0·68	0·29	0·37	5·74	,,	7·59	1·88	,,	0·28	
																1st light 3 a.m.
4 a.m.	53	37·7	2·09	1·44 (?)	+0·65 (?)	1·76	0·15	0·08	0·43	5·70	0·00	5·71	1·42	0·00	1·33	

TABLE II.

Potato Stalks. July 16th–17th, 1914.

Time	Sugars in leaf %		% of stalk soluble in alcohol	Saccharose % on T.V.D.M.				Hexoses as invert sugar %	I.S./C.S.	Pentose %	Pentosan %	Maltose %	Aqueous extract		Soluble starch %	True starch %	
	Saccharose	Hexoses		Citric acid	Invertase	Δ = C.A. − I.	Average						α_D per 100 grms. T.V.D.M. in 100 cc.	α_D calculated as soluble starch %			
6 a.m.	2·14	0·40	35·9	3·20	3·28	−0·08	3·24	4·94	1·52	0·43	12·45	0·00	8·93°	2·21	0·00	0·10	Day
2 p.m.	3·66	0·67	39·7	3·44	3·41	+0·03	3·42	5·58	1·63	0·50	11·15	,,	11·12	2·75	,	0·27	
8 p.m.	2·73	1·22	38·2	3·55	3·60	−0·05	3·57	5·63	1·58	0·53	12·15	,,	5·73	1·42	,,	0·13	
2 a.m.	2·32	0·68	35·2	2·61	2·70	−0·09	2·65	4·63	1·75	0·75	12·10	0·00	7·46	1·85	0·00	0·62	Night

DISCUSSION OF RESULTS.

A. *The Relation between the Sugars and Starch of the Leaf.*

As in the mangold leaf during the early stages of growth, saccharose is the predominating sugar in the potato leaf—the curve of saccharose in Fig. 1 is well above the curve of hexoses and is distinguished from it by its regularity. From 6 a.m. to 2 p.m. as the temperature

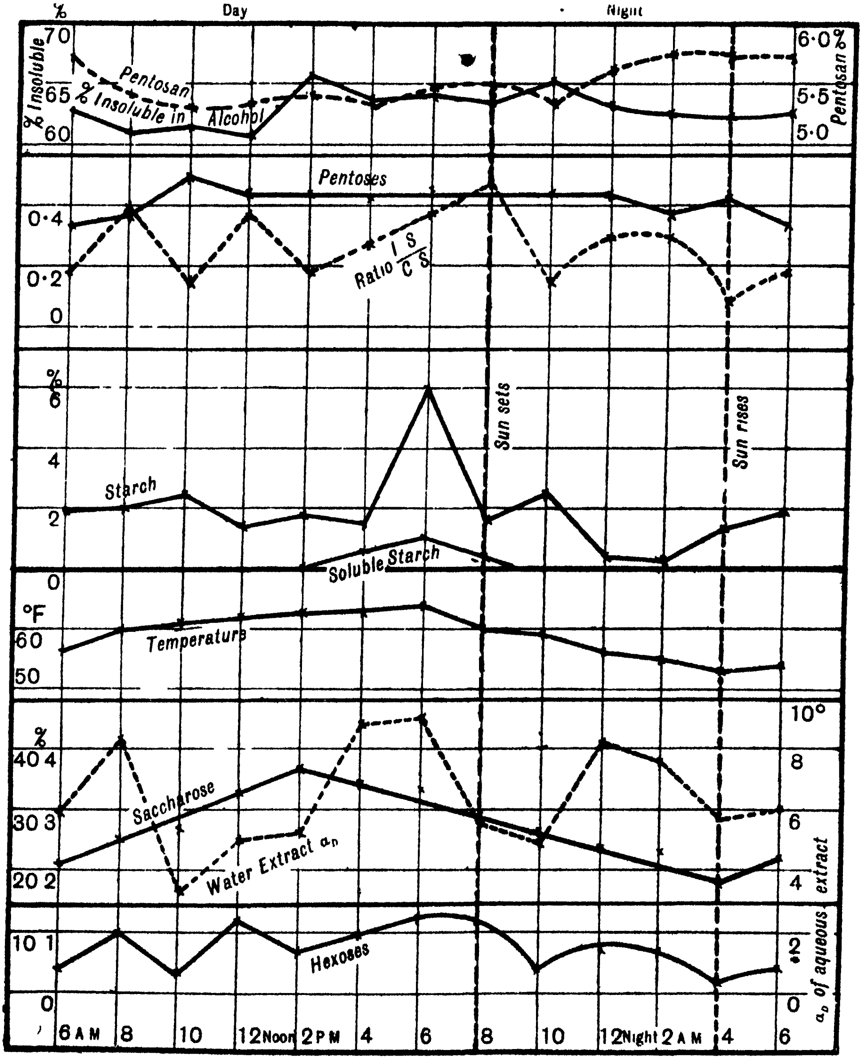

Fig. 1. Potato leaves, July 16–17, 1914.

rises the saccharose increases along practically a straight line, which runs more or less closely parallel to the temperature curve. The maximum of saccharose is, however, reached earlier than the temperature maximum, viz. at 2 p.m.; after this the saccharose falls continuously along nearly a straight line, throughout the rest of the day and night, until sunrise next morning. The range of variation during the 24 hours is from 1·76 to 3·66 per cent.

The hexoses are present in relatively small amount and during the day fluctuate far more, and less regularly, than the cane sugar: the total variation is only from 0·4 to 1·2 per cent. The small changes in the hexoses between 6 a.m. and 2 p.m. synchronise with small changes in the starch present in the leaf, as if interconversion of these substances occurred. As will be seen later, if any reliance can be put upon the dextrose values, it is the dextrose which undergoes the greatest change (see Fig. 3). This sugar appears to fall from 8 a.m. to 10 a.m., whilst the starch increases; from 10 a.m. to noon the starch falls slightly and the dextrose increases. From 12 to 2 p.m. the starch increases and the dextrose falls almost to zero. After 2 p.m. the saccharose steadily falls whilst the hexoses increase, apparently owing to the inversion of the cane sugar, until 8 p.m. At the same time a sudden rise in the starch occurs between 4 p.m. and 6 p.m.; the starch which during the earlier part of the day had changed very little increases from 1·5 to 5·95 per cent. It is a striking fact that directly after the saccharose reaches its maximum at 2 p.m. the "soluble starch" (or dextrin) can be detected in the leaf material. This increases along a straight line until a maximum is reached at 6 p.m. which corresponds with the maximum of the starch. It is probable that the "soluble starch" is formed as an intermediate product between the hexoses (? dextrose) and the true, insoluble starch stored in the leaf. This form of starch is only to be detected in the leaf between 2 p.m. and about 9 p.m., its formation synchronising with the abnormally rapid increase of the starch, which occurs 2 or 3 hours before sunset. In this particular case, the starch stored in the leaf just before sunset is apparently very rapidly put under contribution again, as it falls in amount to about 1·6 per cent. at 8 p.m. The rapid fall of hexoses from 1·2 to 0·4 per cent. between 8 p.m. and 10 p.m. corresponds with a rise of starch from 1·6 to 2·6 per cent., whilst the fall of starch from 10 p.m. to midnight corresponds with a rise of hexoses. Between 12 midnight and 2 a.m. starch has almost disappeared from the leaf (0·2 to 0·3 per cent.), but just before sunrise, apparently in response to the first sign of daylight, the starch increases to about 1·3 per cent., whilst the hexoses fall correspondingly. It is noteworthy that the starch appears to be formed in early morning considerably before the sugars show any increase. The slight increase of starch after sunset, between 8 p.m. and 10 p.m., at the expense of the hexoses is also very striking; at this time of day the intensity of the light was small.

Hexose-saccharose ratio. The curve showing the variation of this ratio naturally follows in its general outline the hexose curve with its abrupt changes. This is a consequence of the linear character of the saccharose curve.

Pentoses. These show a slight increase in the early part of the day, but from noon onwards are practically constant.

Pentosans and matter insoluble in alcohol. In the mangold leaf one of the most striking features was the absolute parallelism of the curves of pentosans and of matter insoluble in alcohol. This parallelism is almost entirely lost in the case of the potato leaf, apparently owing to the presence of starch and its precursors. At night, in particular, the pentosans appear to increase, whilst the matter insoluble in alcohol (including the starch) diminishes.

Rotation of the aqueous extract of the dried leaf tissue left after extracting the sugars. The curve showing the variation of the rotation of the aqueous extract of the dried leaf tissue from which all alcohol-soluble substances have been removed is probably an index of the variation of synthetical products intermediate between the hexoses and starch. Generally speaking this curve is intermediate in its character between the starch curve and the hexose curve. Table 1 shows that the rotation of this extract calculated as soluble starch points to the presence of considerable quantities of substances with a high *positive* rotation, which are possibly of the nature of gums but more probably are up-grade or down-grade products of starch, other than dextrin or soluble starch. They are generally not convertible into hexose by taka-diastase; it is only between 4 p.m. and 8 p.m. that a small quantity of a substance which is so convertible appears in the leaf. Even when this is present the rotation of the aqueous extract is from 2½ to 4 times that corresponding with the "soluble starch" actually found.

During the early part of the day up to 12 noon the curve of the rotation, α_D, is more or less parallel with the hexose curve[1]; as the hexoses rise so does the rotation of the aqueous extract and when the hexoses fall abruptly, as between 8 and 10 a.m., *when the starch increases*, the rotation also falls very greatly. From 10 a.m. to 2 p.m. the general character of the hexose and rotation curves is similar as regards rise and fall; from 2 p.m. onwards, when the cane sugar falls and the hexoses increase, there is a rapid rise of the rotation curve, which seems to follow more or less the formation of "soluble starch" and starch. The rotation curve reaches a maximum at the same time (6 p.m.) as the hexoses, soluble starch and true starch, and then falls abruptly, just as the starch curves fall, between 6 p.m. and 8 p.m. At night the rotation curve follows, on an exaggerated scale, the curve of hexoses and is the inverse of the starch curve.

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[1] It must be borne in mind that the two curves (hexose and rotation curves) apply to different portions of the material analysed: the hexoses are estimated in the alcohol-soluble extract, whilst the rotation curve refers to the *aqueous* extract of the material left after all the substances soluble in alcohol have been removed.

The intimate relation existing between the three curves under discussion, which show the variation of the hexoses, starch and rotation of the aqueous extract of the sugar-free leaf, points to the starch and hexoses being readily interconvertible. The substance with high positive rotatory power which appears so intimately related to the starch and hexoses may either be an intermediate product in the synthesis of starch (other than dextrin or soluble starch) or a substance such as a protein or gum, with a high positive rotation, which stands in close causal relationship with this synthesis. In the present state of our knowledge it is useless to offer further conjectures.

B. *The Stalks and the Translocation of the Sugars.*

As in the mangold stalks, the saccharose remains practically constant in the potato stalk throughout the day (3·2 to 3·6 per cent.) in spite of a much larger variation of this sugar in the leaf (see Fig. 2). At night a slight fall occurs followed by an increase after sunrise to nearly the former level. The hexoses vary in somewhat the same way, but the range of variation is greater during the day (4·94 to 5·63) and the fall at night correspondingly larger (5·63 to 4·63). The curve of *apparent dextrose* (for data see Table V) is almost parallel to the saccharose curve and the same is true of the curve of *apparent laevulose*; the dextrose, as in the mangold stalk, always appears to be in large excess as compared with the laevulose, the ratio $\frac{D}{L}$ (pentoses as xylose) varying between 4·5 and 5·5. Although the laevulose and dextrose curves are practically parallel, the *absolute* increases during the day being nearly the same, the value $\frac{D}{L}$

falls considerably from 6 a.m. to 2 p.m., owing to the smallness of the laevulose values.

In the potato stalks, the fluctuations of the "apparent laevulose" are far less than in the mangold stalks (compare Fig. 2 with Figs. 7, 8 and 9 in preceding paper I), whilst the ratio of hexoses to saccharose remains nearly constant (see Table II) throughout the 24 hours. The variation of "apparent" dextrose and "apparent" laevulose is discussed later (see p. 373).

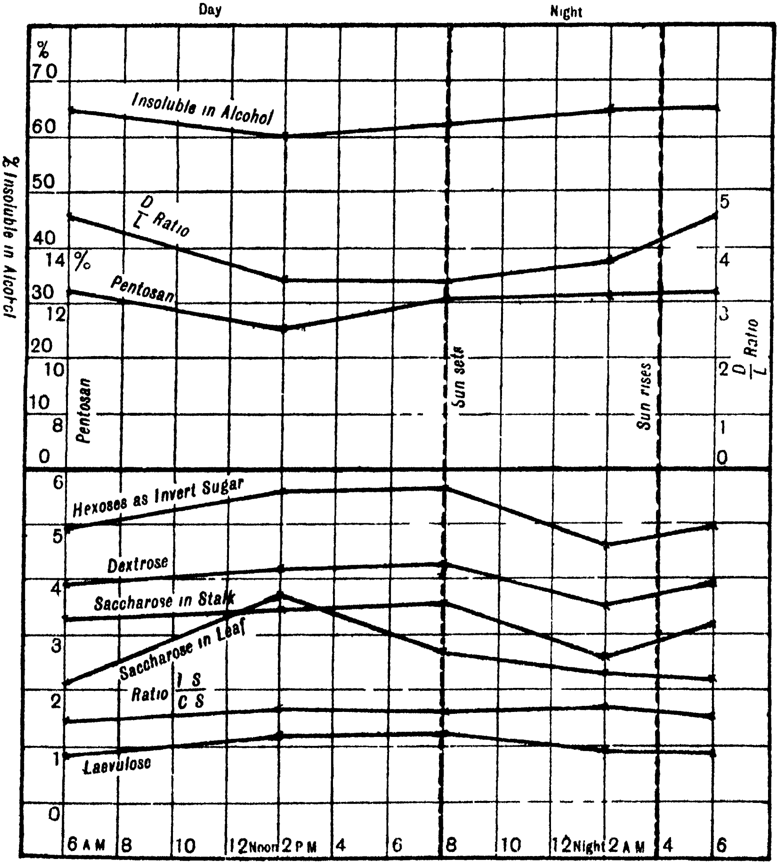

Fig. 2. Potato stalks, July 16–17, 1914.

The fact that in the leaf the saccharose is always greatly in excess of the hexoses (ratio $\frac{\text{I.S.}}{\text{C.S.}}$ varies from 0·08 to 0·45) whereas in the stalks the hexoses are always greatly in excess of the saccharose (ratio $\frac{\text{I.S.}}{\text{C.S.}}$

varies from 1·52 to 1·75) is best explained as in the case of the mangold by the view that in the leaf the saccharose is a primary product and is converted into hexoses for purposes of translocation.

Pentosans and Matter Insoluble in Alcohol.

In the stalks of the potato, unlike the leaves, practically no starch is present to interfere with the parallelism of the curves showing the pentosan content and the total leaf matter insoluble in alcohol (cellulose + lignified tissue) (see Fig. 2). From 6 a.m. to 2 p.m. the sugars and other substances soluble in alcohol are increasing so that the proportion of matter insoluble in alcohol falls. It should be noted that in the potato stalks the sugars form only a small proportion of the increase of total soluble matter; thus between 6 a.m. and 2 p.m. their increase is only 0·8 per cent., whilst the other soluble matters increase by 3·8 per cent.[1] After 2 p.m. the insoluble matter gradually increases to practically its earlier value, and the same is true of the pentosans.

The following table (Table III) gives a comparison between the potato and the mangold as regards the range of variation of the total sugars and of the matter soluble in alcohol in the leaf and stalk.

As regards the *leaf* constituents this table shows that the potato in its early stages of growth closely resembles the mangold at a corresponding stage; the range of variation of the sugars and of the substances soluble in alcohol is nearly the same in both cases. In both cases also the saccharose is greatly in excess of the hexoses. It is probable that, in the potato as in the mangold, during the later period of growth, when storage is the principal function, the relative proportior of saccharose and hexoses would be found to change, the hexoses then predominating in the leaf as well as in the stalks.

In the potato *stalks*, however, the actual proportion of substances soluble in alcohol is considerably less (35·2–39·7) than in the mangold (43·4-46·8), but the range of variation during the day is greater. The variation of the sugars (1·93 per cent.) is however far less than in the mangold (4·68). The last two columns show how greatly the proportion of sugars and substances soluble in alcohol increases in the mangold in the later stages of growth.

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[1] In the mangold stalks during the day the increase of the sugars is considerably greater than the increase of the total substances soluble in alcohol; during this period the soluble substances other than the sugars (amino-acids, tannins, amides) fall off greatly relatively to the sugars. Thus:

Mangold Stalks. Series I. August 26th–27th (average of top and bottom halves).
Increase of total sugars from 6 a.m. to noon = 4·87 %
Increase of total alcohol-soluble substances = 3·0 %

Mangold Stalks. Series II. September 10th–11th.
Increase of total sugars from 10 a.m. to 4 p.m. = 6·20 %
Increase of total alcohol-soluble substances = 1·5 %

TABLE III.

Range of Variation of Sugars and Alcohol-soluble Matter in the Mangold and Potato.

	Potato July 16–17, 1914	Mangold Series I, Aug. 26–27, 1913	Mangold Series II, Sept. 10–11, 1912	Mangold Series III, Oct. 11–12, 1912
Leaf:	%	%	%	%
Total sugars	1·91–4·93 Δ = 3·02	1·70–5·27 Δ = 3·57	9·62–17·17 Δ = 7·55	14·5–21·0 Δ = 6·5
Alcohol-soluble substances	34·1–39·3 Δ = 5·2	37·2–42·5 Δ = 5·3	44·2–54·7 Δ = 10·5	47·9–54·95 Δ = 7·05
Stalks:				
Total sugars	7·28–9·21 Δ = 1·93	10·95–15·63 Δ = 4·68	25·32–31·76 Δ = 6·44	— —
Alcohol-soluble substances	35·2–39·7 Δ = 4·5	43·4–46·8 Δ = 3·4	64·2–66·9 Δ = 2·7	— —

Table III shows that in both mangold and potato *leaves* the daily fluctuation of the substances soluble in alcohol is always far greater than (often nearly double) that of the total sugars. The same is true of the potato *stalk*, but in the mangold stalk the change in the sugars is always much greater than that of the alcohol-soluble constituents.

C. *The Dextrose-Laevulose Ratio.*

The "apparent" dextrose and laevulose have been calculated, as in the case of the mangold, on the assumption that the pentoses are either arabinose or xylose. The values are given in Tables IV and V.

D = percentage of apparent dextrose calculated on the total vacuum-dried matter (T.V.D.M.).

L = percentage of apparent laevulose calculated on the total vacuum-dried matter (T.V.D.M.).

I. *Leaves.*

As was the case in Series I of the mangold pickings (Paper II, p. 335) the results obtained for the "apparent" dextrose and laevulose are of little real value as an index of the true proportions of these sugars present, owing to the presence of optically active impurities which cannot be removed by the ordinary treatment with basic lead acetate. The quantity of the reducing sugars is so small that the error introduced in this way becomes relatively very great; a large difference too is seen between the results for dextrose and laevulose according as the pentose is assumed to be arabinose or xylose. This is not because the pentose is actually present in large amount (it ranges only from 0·35 to 0·52

TABLE IV.

Apparent Dextrose and Laevulose in Potato Leaves.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%		Hexoses % calc. as invert sugar	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	Day
8 a.m.	0·26	0·76	0·34	0·46	0·53	0·85	1·01	1·00	1·00	
10 a.m.	Nil	0·38	0·00	Nil	0·38	0·00	0·38	0·38	0·37	
12 noon	0·25	0·95	0·26	0·48	0·73	0·66	1·23	1·21	1·21	
2 p.m	Nil	0·69	0·00	Nil	0·69	0·00	0·69	0·69	0·67	
4 p.m	Nil	0·97	0·00	Nil	0·97	0·00	0·97	0·97	0·93	
6 p.m.	0·19	1·11	0·17	0·45	0·83	0·54	1·30	1·28	1·27	
8 p.m.	0·17	1·08	0·16	0·39	0·83	0·47	1·25	1·22	1·22	
10 p.m.	Nil	0·42	0·00	Nil	0·42	0·00	0·42	0·42	0·40	Night
12 night	Nil	0·76	0·00	0·18	0·57	0·32	0·76	0·75	0·73	
2 a.m.	Nil	0·71	0·00	0·19	0·50	0·38	0·71	0·69	0·68	
4 a.m.	0·01	0·15	0·06	0·15	Nil	∞	0·16	0·15	0·15	

TABLE V.

Apparent Dextrose and Laevulose in Potato Stalks.
July 16th–17th, 1914.

Time	Pentose as arabinose			Pentose as xylose			$D+L$%		Hexoses % calc. as invert sugar	
	D%	L%	$\frac{D}{L}$	D%	L%	$\frac{D}{L}$	Pentose as arabinose	Pentose as xylose		
6 a.m.	3·68	1·11	3·32	3·91	0·86	4·58	4·79	4·77	4·94	Day
2 p.m.	3·90	1·53	2·55	4·17	1·23	3·39	5·43	5·40	5·40	
8 p.m.	3·95	1·55	2·55	4·23	1·24	3·41	5·50	5·47	5·63	
2 a.m.	3·25	1·25	2·60	3·53	0·94	3·75	4·50	4·47	4·63	Night

per cent. on the T.V.D.M.), but because the quantity and rotatory power of the hexoses is exceedingly small (0·15 to 1·27 per cent.). At 8 a.m., for instance, if the pentoses are taken as arabinose, $\frac{D}{L} = 0{\cdot}34$, but if they are assumed to be xylose $\frac{D}{L}$ becomes 0·85.

That the results are vitiated by the presence of a *laevo*-rotatory impurity appears clearly in the data for 6 a.m., 10 a.m., 2 p.m., 4 p.m. and 10 p.m., in all of which cases the amount of dextrose appears to be *nil*. If in these cases the whole of the reducing sugar is assumed to be laevulose, the negative rotation calculated does not account, on the assumption that the pentose is xylose, for the negative rotation actually observed. The following table shows the differences:

Time	Actually observed for hexoses in 200 mm. tube at 20°*	Calculated for hexose = laevulose	Rotation not accounted for
6 a.m.	−0·143	−0·060°	−0·083°
10 a.m.	−0·076	−0·036	−0·040
2 p.m.	−0·075	−0·055	−0·020
4 p.m.	−0·146	−0·102	−0·044
10 p.m.	−0·063	−0·039	−0·024

* After allowing for the pentoses (as xylose) and saccharose present

If the results are calculated on the assumption that the pentose is arabinose, the negative rotation not accounted for becomes even greater; thus at 6 a.m. it becomes − 0·138° instead of − 0·083°. The number of cases in which dextrose appears to be entirely absent is increased on this assumption.

It is clear therefore that the apparent predominance of laevulose in the potato *leaf* is due to the presence of relatively large quantities of a laevo-rotatory impurity (? asparagine), and it is probable that the dextrose and laevulose, as in the mangold leaf, are really present in equal proportions, that is as invert sugar, and are formed from saccharose. It is interesting that the dextrose appears in largest amount at 6 p.m. and 8 p.m., that is at the time when the starch content reaches a maximum and is subsequently put under contribution. As we show on p. 357, the starch is broken down by the leaf enzymes completely to dextrose. Fig. 3 shows the variation of the apparent dextrose and laevulose during the 24 hours (pentoses assumed to be xylose); as an index of the real fluctuation of the hexoses the curves have, of course, no value, but they are interesting as showing that the laevo-rotatory impurity varies regularly during the 24 hours. The laevulose always appears in considerable excess except just about sunrise (4 a.m.) when the whole of the very small amount of hexose present (0·15 per cent.) appears as *dextrose* not laevulose, and a *positive* rotation of + 0·015 remains unaccounted for. A somewhat similar abnormality was found just before sunrise in the case of the mangold leaves, Series I; whereas during the greater part of the 24 hours *dextrose* appeared to be in excess (see Table I, Paper II, p. 332, arabinose values) in the mangold leaf, at 4 a.m., when the total hexose was exceedingly small (0·2 per cent.), laevulose suddenly appeared to predominate; at the same time, the polarisation results for cane sugar, which in general throughout the day were *higher* than the reduction values, suddenly became *lower*. But 2 hours later, at 6 a.m., the values were again much higher. The following data illustrate this:

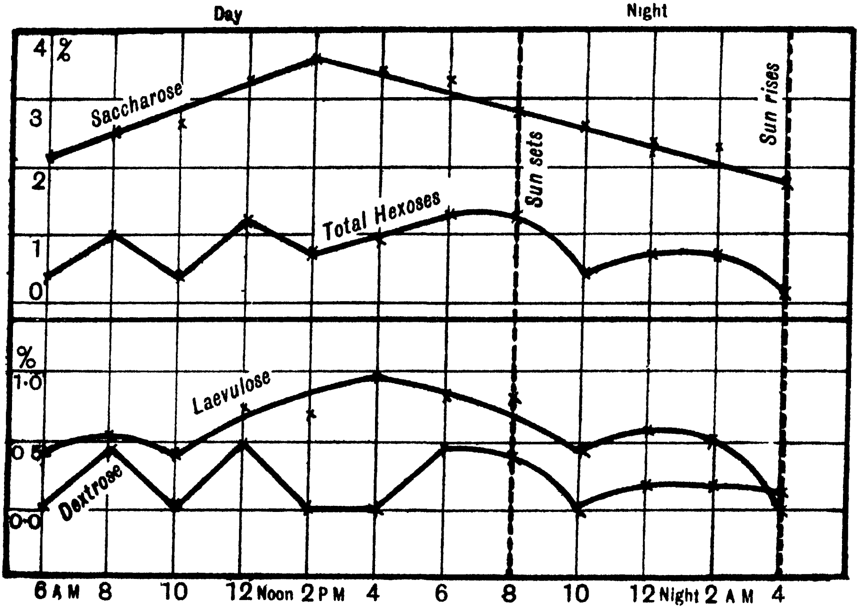

Fig. 3. Potato leaves, July 16–17, 1914, dextrose and laevulose (apparent). (Pentose as xylose.)

Mangold Leaves. Series 1. August 26th–27th, 1913

	D %	*L* %	$\frac{D}{L}$	Δ* in saccharose values
2 a.m. ...	0·29	0·08	3·62	+ 7·3
4 a.m. ...	0·00	0·20	0	- 29·2
6 a.m. ...	0·73	0·00	∞	+ 42·6

Sunrise = 5.5 a.m

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* Δ = difference between values found for saccharose by polarisation and by reduction.

It would appear, therefore, that in the potato as in the mangold leaf *two* oppositely active impurities are present at different times of the day. During the greater part of the day laevulose appears to be in excess owing to a laevo-rotatory impurity predominating, but *at night* the amount of this impurity diminishes until it is replaced just before sunrise (4 a.m.) by an excess of *dextro*-impurity. The variation is well seen by considering the data obtained by assuming the pentoses to be arabinose; the amount of laevo-rotation left unaccounted for when the whole of the hexose is assumed to be laevulose gradually drops from 10 p.m. to 2 a.m., whilst at 4 a.m. dextrose appears to be present.

At 10 p.m. negative rotation unaccounted for = −0·062° (200 mm. tube)
12 midnight negative rotation unaccounted for = −0·010°
2 a.m. negative rotation unaccounted for = −0·002°
4 a.m. positive rotation unaccounted for = +0·015°

Between 6 a.m. and 8 a.m., that is just after sunrise, the quantity of *negative* impurity suddenly increases very largely, the negative reading unaccounted for at 6 a.m. being greater than at any other period of the 24 hours (− 0·138° if pentose is arabinose, − 0·083° if xylose).

The following table (Table VI) shows how the presence of the optically active impurities causes abnormally large differences in the results found for saccharose by the reduction and by the polarisation methods. This table should be compared with the similar table obtained in the case of the mangold leaf (see p. 338, preceding paper).

TABLE VI.

Divergence of Results for Saccharose by the Reduction and Polarisation Methods—Potato Leaves. July 16th–17th, 1914.

	Citric acid inversion			Invertase inversion			
Time	% saccharose reduction	% saccharose polarisation	Δ%	% saccharose reduction	% saccharose polarisation	Δ%	$\frac{D}{L}$ (pentose xylose)
6 a.m.	2·16	2·48	+14·8	2·11	2·70	+17·1	0·00
8 a.m.	2·47	2·37	− 4·0	2·60	2·67	+ 2·7	0·85
10 a.m.	2·81	2·71	− 3·5	2·65	3·10	+17·0	0·00
12 noon	3·39	3·63	+ 7·1	3·19	3·41	+ 6·9	0·66
2 p.m.	3·81	4·46	+17·0	3·50	4·54	+29·7	0·00
4 p.m.	3·56	3·75	+ 5·3	3·34	3·83	+14·6	0·00
6 p.m.	3·46	4·34	+25·4	3·22	4·48	+39·2	0·54
8 p.m.	2·77	2·21	−20·2	2·69	2·98	+10·8	0·47
10 p.m.	2·76	2·31	−16·2	2·49	2·77	+11·2	0·00
12 night	2·48	2·46	− 0·8	2·30	2·39	+ 3·9	0·32
2 a.m.	2·38	1·91	−19·8	2·26	1·98	−12·4	0·38
4 a.m.	2·09	2·43	+16·2	1·44	2·51	+74·4	∞

As in the case of the mangold leaves, the polarisation results for saccharose are generally considerably *higher*—often 20 to 30 per cent. higher—than the reduction values[1]. The two methods only give approximately the same results when $\frac{D}{L}$ approximates to 1 (e.g. at 8 a.m. and 12 noon, when Δ (invertase) is only 2·7 and 6·9 per cent.). When the dextrose appears to disappear $\left(\frac{D}{L}=0\right)$ the divergences (Δ) are greatest (e.g. 6 a.m., 10 a.m., 2 p.m., 4 p.m., 10 p.m.). As showing the presence of *two* distinct and oppositely active impurities, it is interesting that between 6 a.m. and 4 p.m. when the laevulose on the whole appears to increase (see Fig. 3) a rise in the apparent dextrose corresponds with a diminution in the difference between polarisation and reduction values for saccharose, and *vice versa*; but from 4 p.m. to 10 p.m. (laevulose falling) an increase in the apparent dextrose carries with it an increase in the divergence, whilst a fall in the dextrose is accompanied by an opposite result (compare the figures in Table VI with the curves in Fig. 3). Between 10 p.m. and 2 a.m., when the laevulose again appears to rise and fall, the difference between the two sets of values becomes less and less (invertase figures) and finally *negative*. It is interesting to compare the following figures:

				Sunrise	
	10 p.m.	Midnight	2 a m.	4 a.m.	6 a.m
Rotation not accounted for by the hexoses ...	−0·062°	−0·010°	−0·002°	+0·015°	−0·081°
Δ (invertase) between saccharose values ...	+11·0 %	+ 3·8 %	−12·5 %	+74 %	+17·2 %

The abrupt change between 2 a.m. and 4 a.m. (corresponding with the sudden fall to zero of the apparent laevulose) from a negative rotation not accounted for to a high positive value, and a negative difference − 12·5 per cent. to a high positive value + 74 per cent., is followed, *immediately after sunrise*, by equally great changes in the opposite direction. The fluctuations, whatever be their cause, show throughout evidences of periodicity; this appears most clearly in the shape of the curve of apparent laevulose.

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[1] If the discrepancy between the results for saccharose were due solely to an amino-acid or amide such as asparagine, one would expect the divergence to be diminished by taking the first (direct) polarisation *after* saturating the solution with sulphur dioxide so as to make it strongly acid (see Pellet, *Dosage du Sucre par Inversion*, 1913). As a matter of fact in the case of the potato whether the direct reading was taken (as has been usual in our experiments) in practically neutral solution or whether it was taken in acid solution (SO_2) made very little difference in the majority of cases, the figures usually being very high as compared with the reduction values.

II. *Stalks.*

The results for the potato stalks closely resemble those found for the mangold stalks in the fact that the dextrose present appears always to be in large excess as compared with the laevulose; the ratio $\frac{D}{L}$ varies from 3·39 to 4·58 (pentose as xylose). But there is this striking difference: in the mangold, dextrose appeared to be the predominant sugar in both leaf and stalks, but in the potato it is in excess *only in the stalks*, whilst in the *leaf*, as pointed out above, laevulose predominates. It is also very striking, that whereas in the mangold the greatest fluctuations and the greatest divergences between the reduction and polarisation values for saccharose were found in the stalks and mid-ribs (Δ varied from + 40 per cent. to − 90 per cent., see Table VIII, preceding paper), caused no doubt by large fluctuations in the optically active impurities present, in the potato stalks *the differences are as a rule relatively small, and, in general, less than in the leaves.*

The following table (Table VII) shows this:

TABLE VII.

Divergence of Values of Saccharose by Polarisation and Reduction Methods—Potato Stalks. July 16th-17th, 1914.

	Citric acid inversion			Invertase inversion			
Time	Saccharose by reduction %	Saccharose by polarisation %	Δ %	Saccharose by reduction %	Saccharose by polarisation %	Δ %	$\frac{D}{L}$ (xylose
6 a.m.	3·20	3·70	+15·6	3·28	3·62	+10·4	4·58
2 p.m.	3·44	3·84	+11·6	3·41	3·92	+14·9	3·39
8 p.m.	3·55	3·35	− 5·6	3·60	3·85	+ 7·0	3·41
2 a.m.	2·61	2·82	+ 8·0	2·70	2·88	+ 6·7	3·75

The extreme divergence here is only 15 per cent., whilst in general the divergence (Δ) is less than 10 per cent. There are no such abrupt changes from positive values to negative values as were met with in the mangold stalks (see p. 344) and had their counterpart in the sudden variation in the values for apparent laevulose (see Figs. 7 and 8, Paper I). One of the most striking differences between the potato stalks and the mangold stalks is that in the former the curves *of apparent dextrose and apparent laevulose run almost parallel to one another* throughout the 24 hours, and at the same time about parallel to the saccharose curve (see Fig. 2, p. 371). Each sugar increases slightly and continuously during the day and then falls at night.

It seems probable from the parallelism of the curves of saccharose and total hexoses that the dextrose and laevulose are actually present in the stalks as invert sugar, being formed from the saccharose by inversion; the large apparent excess of dextrose would then be due to the presence of a *dextro*-rotatory impurity which accumulates in the stalks (whereas in the leaf a *laevo*-rotatory substance is generally in excess). The divergence Δ between the reduction and polarisation values is relatively small in the case of the potato because the substance is of such a nature that the *change* of rotation brought about by the processes of inversion is relatively small; but the existence of this divergence (up to 15 per cent.) is a proof that some such compound is present. On the other hand the practical parallelism of the curves of apparent dextrose and laevulose, which is in striking contrast with the *mangold* stalks, suggests that whatever be the impurity which is present, its amount remains relatively constant throughout the 24 hours.

On the other hand the relatively small divergence between the polarisation and reduction values for saccharose in the potato stalks, in contrast with the large divergences found in the mangold stalks, may be taken to mean that only small amounts of the optically active impurity are present in the potato stalks and that the values of dextrose and laevulose in the stalks (*not* in the leaves) nearly represent the true values for these sugars. If this is the case the dextrose has accumulated in the stalks far more than the laevulose, possibly owing to the latter sugar being used up for tissue building[1], and to the fact that the starch formed in the leaf gives dextrose as sole product when hydrolysed by the leaf enzymes (see p. 357). One would naturally expect the starch in the tuber to be built up from dextrose as it yields dextrose exclusively when hydrolysed by either acids or taka-diastase and the predominance of dextrose in the stalks conveying sugars to the tuber would be quite natural if this were the case. The question, however, whether the dextrose is in actual excess over the laevulose in the stalks or whether the two sugars are present mainly in the form of invert sugar can only be decided definitely when methods have been devised of estimating the two sugars, in presence of each other, which are free from the errors caused by optically active impurities.

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[1] It is interesting to recall Meyer's observation in 1886 that almost all leaves capable of forming starch at all produce it abundantly from a 10 per cent. solution of laevulose and a relatively small number only from dextrose. On general grounds, considering the relationship of starch and dextrose, one would have expected the reverse to be the case.

SUMMARY.

1. In the potato leaf when the tubers are beginning to develop the principal sugar present is saccharose; its amount increases from sunrise up to 2 p.m., following approximately the curve of temperature. It then falls during the rest of the day and night. The rise and fall are both linear.

2. The hexoses are present in the leaf in very small amounts—generally less than 1 per cent. of the total dry weight of the leaf. They fluctuate considerably during the early part of the day, the fluctuations being apparently determined by conversion into or formation from starch.

3. During the early part of the day up to 2 p.m. the proportion of starch changes very little, the small fluctuations which occur being related to changes in the starch. The starch is apparently formed from the hexoses.

4. Directly the amount of saccharose has reached its maximum at 2 p.m. the hexoses begin to increase in the leaf, owing apparently to hydrolysis of the saccharose to invert sugar; at the same time "soluble starch" (or dextrin) is first detected in the leaf and its amount increases regularly up to 6 p.m. At 6 p.m., 2 hours before sunset, the true starch in the leaf reaches a maximum value, far greater than any previous value during the day. The starch and "soluble starch" subsequently fall rapidly until between midnight and 2 a.m. the amount left is exceedingly small (0·2 per cent.). The starch is apparently converted directly into hexose (dextrose), the amount of which increases in the leaf.

5. In the stalks reducing sugars predominate greatly over the saccharose in spite of the fact that in the leaf the latter is in excess. As in the mangold it is probable that cane sugar is the first sugar formed in the leaf and that it is hydrolysed by invertase in the veins, mid-ribs and stalks, for the purpose of translocation.

6. As in the mangold, the true proportions of dextrose and laevulose cannot be determined in the leaves and stalks owing to the presence of soluble optically-active impurities, which vitiate the polarimetric data.

It is shown that the presence of these impurities also falsifies the results obtained for saccharose by the double polarisation method. The fluctuations of the "apparent dextrose" and "apparent laevulose" in the leaf really indicate variations in these impurities rather than variations in the hexoses, which are perhaps present mainly as invert sugar. In the stalks, where the amount of optically active impurity appears to be less than in the leaves, it is possible that the dextrose is actually in excess as it appears to be, and that the starch in the tuber is built up from this sugar.

7. Maltose is invariably absent from the potato leaf and also from the leaves of other plants which form much starch in the leaf. The degradation of starch in the leaves is probably effected by a mixture of enzymes similar to the enzymes of *Aspergillus oryzae* (taka-diastase); maltase is always in relative excess, so that the starch is degraded completely to dextrose. The series of changes is therefore:

Starch → dextrins → maltose → dextrose.

APPENDIX. EXPERIMENTAL DATA.

Potato Leaves. July 16th–17th, 1914.

In the first two analyses (6 a.m. and 8 a.m.), after the treatment with basic lead acetate, the precipitate was washed to 2 litres, but the first and second litre of washings were analysed separately so as to obtain an idea of the amount of sugars left behind when the washing was continued only to 1 litre. As it was found that this was quite appreciable, in all the later analyses the lead precipitate was washed to nearly 2 litres, and after precipitation with sodium carbonate the solution was made to 2000 cc. (= A).

The extract of the leaf material was evaporated *in vacuo* and made up to 500 cc. 440 cc. of the 500 were treated with basic lead acetate and the precipitate washed to 1 litre; the filtrate was treated with solid sodium carbonate and made to 1000 cc. = A.

The second litre of washings was also treated with solid sodium carbonate and made up to 1000 cc. = A'.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (x)	Polarisation of *A* in 200 mm. tube*, $\alpha_D^{20^\circ}$	Reduction of x cc. of solution *A*, grms. CuO	Invertase inversion: Reduction of x cc. after inversion, grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. after inversion, grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*	Remarks
6 a.m.	34·32	57·91	92·23	25 cc. *A*	+0·115°	0·0426	0 1445	-0·104°	0·1470	-0·108°	0·0169	1st litre (*A*
	"	"	"	25 cc. *A′*	±0·000	0·0032	—	—	0·0125	-0·000	—	2nd litre (*A*
8 a.m.	43·30	67·55	110·85	25 cc. *A*	+0·261	0·0835	0·2300	-0·075	0·2223	-0·050	0·0127	1st litre (*A*)
	"	"	"	25 cc. *A′*	-0·002	0·0024	—	—	0·0190	-0·022	—	2nd litre (*A′*
10 a.m.	44·15	70·58	114·73	25 cc. *A*	+0·117	0·0387	0·1247	-0·078	0·1298	-0·061	0·0077	*A* = 2000 cc
12 noon	44·75	69·24	113·99	25 cc. *A*	+0·183	0·0513	0·1548	0·056	0·1612	-0·066	0·0054	"
2 p.m.	34·57	66·72	101·29	25 cc. *A*	+0·149	0·0312	0·1318	-0·051	0·1408	-0·050	0·0045	"
4 p.m.	49·38	87·17	136·55	25 cc. *A*	+0·139	0·0505	0·1800	-0·096	0·1887	-0·100	0·0071	"
6 p.m.	43·22	77·50	120·72	25 cc. *A*	+0·189	0·0578	0·1684	-0 107	0·1767	-0·101	0·0069	,
8 p.m.	59·25	102·42	161·67	25 cc. *A*	+0·190	0·0725	0 1960	-0·090	0·1998	-0·042	0·0094	"
10 p.m.	39·74	74·30	114·04	25 cc. *A*	+0·120	0·0272	0·1073	-0·061	0·1161	-0·041	0·0059	"
Midnight	43·87	76·07	119·94	25 cc. *A*	+0·133	0·0390	0·1168	-0·044	0·1232	-0·047	0·0063	"
2 a.m.	53·22	88·90	142·12	25 cc. *A*	+0·168	0·0415	0·1328	-0·024	0·1375	-0·020	0·0063	"
4 a.m.	42·58	70·36	112·94	25 cc *A*	+0·147	0·0188	0·0648	-0·035	0·0857	-0·032	0·0053	"

* In most cases the solution was sufficiently colourless to allow the reading to be taken in a 400 mm. tube. The data given are, however, all reduced to the 200 mm. standard

Potato Stalks. July 16th–17th, 1914.

Distilled *in vacuo* and made up to 250 cc. 190 cc. of the 250 treated with basic lead acetate, filtered and washed to 1 litre, solid sodium carbonate added and made up to 1000 cc. = Solution *A*.

Time	Vacuum-dried matter soluble in alcohol, grms.	Vacuum-dried matter insoluble in alcohol, grms.	Total vacuum-dried matter, grms.	Volume of solution *A* used for reduction (x)	Polarisation of *A* in 200 mm. tube, $\alpha_D^{20^\circ}$	Reduction of x cc. of solution *A*, grms. CuO	Invertase inversion: Reduction of x cc after inversion. grms. CuO	Invertase inversion: α_D after inversion (200 mm.)	Citric inversion: Reduction of x cc. after inversion, grms. CuO	Citric inversion: α_D after inversion (200 mm.)	Phloroglucide from 50 cc. *A*
6 a.m.	31·47	56·24	87·71	25 cc. *A*	+0·468°	0·2200	0·3563	+0·023°	0·3525	+0·019°	0·0090
2 p.m.	29·27	44·45	73·72	25 cc. *A*	+0 385	0·2094	0·3292	+0·001	0·3304	+0·005	0·0085
8 p.m.	29·79	48·14	77·93	25 cc. *A*	+0 422	0·2242	0·3568	+0·012	0·3550	+0·038	0·0102
2 a.m.*	25·10	46·14	71·24	25 cc. *A*	+0·345	0·1914	0·2950	+0·021	0·2918	+0·023	0·0170

* In this analysis the extract was made to 200 cc. (not 250) and 170 cc. of the 200 treated with basic lead acetate.

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(*Received October* 4, 1915.)

VOLUME VII APRIL, 1916 PART IV

THE SOILS AND CROPS OF THE MARKET-GARDEN DISTRICT OF BIGGLESWADE.

BY THEODORE RIGG, B.A., *St John's College.*

(*School of Agriculture, Cambridge.*)

INTRODUCTION.

WITHIN the last two or three years the attention of the agricultural world has been directed to the importance of the correlation of the chemical and physical properties of soils with the crops and systems of agriculture which farmers have found most suitable to them.

By such correlations many facts of supreme interest have been elicited. The American Bureau of Soils have been able to enumerate the characteristics and properties of various soil formations which render them suitable for some particular crop. They have been able to suggest new and more profitable crops as likely to be suitable for soils which have not hitherto grown them. They have been able to explain causes of fertility and infertility which have a practicable bearing not only on the agriculture of their own country but of the whole world.

Hall and Russell[1] were similarly able to demonstrate the value of a soil and crop survey to agriculture as a whole, and particularly to that of the district under their investigation. As a result of their work, they were able to give much advice on the improvement of methods of agriculture, mixtures of artificial manures suitable for the crops on each soil formation, the value of liming, etc.

Perhaps as a work of reference, a soil survey is of the greatest and most permanent value, for if the soils of a district have been classified and their properties stated in terms of mechanical and chemical analyses it becomes possible to compare abnormal soils with the normal soil type and to detect more readily the cause of its abnormality.

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[1] Hall and Russell, *Agriculture and Soils of Kent, Surrey and Sussex.* Published by the Board of Agriculture and Fisheries, 1911.

Although soil surveys are in progress in many counties of England, and although much valuable information concerning the chemical and physical properties of different soil formations which make them suitable for ordinary cereal and root crops has been obtained, yet no similar work has been done on the market-garden soils and crops which play so important a part in the agriculture of such a county as Bedfordshire.

The area under market-garden crops increases yearly, and as there is a tendency for small farmers without experience to take up this particular branch of farming, it becomes more and more imperative that such correlations between crops, methods of agriculture, and chemical and physical properties of the soil should be made, in order to supply the inexperienced market-gardener with information concerning the most suitable crops and best systems of treatment and manuring for any soil formation of known properties.

Further, the minute survey of the soils necessary to achieve this end, in a district where there are great "quarternary" accumulations which vary considerably in texture, should throw some light upon the methods of soil survey applicable to counties where there is much "drift" deposit.

At the suggestion of Professor Wood, the writer decided to conduct a detailed examination of the soils and crops of the Biggleswade market-garden area of Bedfordshire. This area is roughly one of 100 square miles, and extends from Henlow in the south to St Neots in the north. Gamlingay marks the eastern limit and perhaps Willington marks an ill-defined western limit to the district. The Great Northern Railway runs through the centre of the district from north to south, giving St Neots, Tempsford, Sandy, Biggleswade, Langford, and Henlow direct connection with London, and communication with the great northern industrial centres, while the London and North-Western Railway crosses the Great Northern Railway almost at right angles and links up Gamlingay, Potton, Sandy, Girtford, Blunham, and Willington with the Midland Railway service to London and midland centres, map 1.

The district is characterized by extensive valley gravel deposits on either side of the Ivel and the Ouse. The Ivel flows almost due north and south, making a slight deviation around the western extremity o' the greensand escarpment at Sandy, and joins its waters with the Ouse at Tempsford. The whole valley system of the Ouse and Ivel is enclosed by boulder clay elevations on the east and west.

The market-garden area proper can be said to lie within the limits of a two mile boundary on either side of the railway, but the writer has gone rather farther afield in order to compare the soils of the immediate vicinity with those used for intensive market-garden culture. This investigation has, however, been primarily concerned with the market-garden soils, and therefore one or two heavy soil formations not suitable for the growth of market-garden crops but included in the investigation have not received perhaps the same detailed examination as those on which market-garden crops are extensively grown.

The investigation may be conveniently divided into three parts. Part I dealing with the methods employed in soil mapping and the relationship of the soil formations to the underlying geological formations. Part II a description of the properties and agriculture of the soil formations and a definition of their properties in terms of chemical and mechanical analyses. Part III the relationship of crops to the soil formations.

PART I.

Methods of Soil Mapping, Soil Definitions and Relationship of the Soil Formations to the Surface Geology.

In conducting any soil survey the first question which arises is the means of distinguishing between different soil types.

The American Bureau of Soils in their extensive surveys have classified soils in "series" according to their structure and colour. Under the "series" name will occur soils which have the "series" characteristic but yet which differ widely in texture. Thus the well-known Miami Series is characterized by the light colour of the surface soils and their derivation from glacial material. There are fourteen members of this series and the variation in texture is from gravel and sand to a clay loam Thus geological origin largely decides series, but the position in the series, whether the conventional terms sand, gravel, clay, etc., should be applied to the soil, is determined by mechanical analysis.

Hall and Russell in their survey of Kent, Surrey and Sussex used the geological map as a basis on which to work in mapping soil formations. In this region the soils are comparatively uniform over an entire geological outcrop. In some instances, notably the Lower Wealden Beds, a greater variation in texture was found but the soils all had certain features in common, the high percentage of silt being particularly noticeable. Owing to the circumstance that this formation possesses little agricultural interest, Hall and Russell made only a very rough division into areas of different texture.

In the market-garden district dealt with in this paper there are large areas where the texture of the original soil has been greatly altered by alluvial wash; there are very extensive valley gravel deposits which are subject to varying texture and structure; and lastly boulder clay deposits give rise to soils in some cases quite different from the majority of boulder clay soils.

Since market-garden crops, as will afterwards be shown, are susceptible to even slight variations in soil texture, it became necessary to map out the extent of all variations occurring on the geological formation before any correlation of crop and soil could be attempted.

The writer has followed Hall and Russell in using the geological formation to mark the extent of a series of soils which have a somewhat similar mineral structure. These series of soils have, however, then been separated into soil formations having different agricultural properties and the extent of each has been mapped.

For the purpose of mapping the soil formations, the six inch Ordnance Survey maps, which show the fields, were used. Every field was examined in a detailed way over the whole area. Field observations of the colour, texture, the agricultural operations and general surface features were used in determining the extent of each soil formation. The six inch maps were then reduced to the one inch scale from which map 4 was constructed showing the various soil formations in the district.

The following geological formations were examined and the series of soils occurring on them were divided into soil formations which had different agricultural properties:

(1) Oxford clay giving rise to two soil formations:
  (*a*) Pure clay soil,
  (*b*) A clay loam probably resulting from an alluvial wash on to the clay soil.

(2) Greensand giving rise to two soil formations:
  (*a*) Dark sands,
  (*b*) Brown sands.

(3) Gault giving rise to two soil formations:
  (*a*) Pure clay soil,
  (*b*) A sandy loam locally known as "Redland," occurring as a narrow strip between greensand and the pure gault clay soil (*a*).

(4) Boulder clay giving rise to three soil formations:
  (*a*) Pure boulder clay soil,
  (*b*) Heavy loam produced by wash on boulder clay,
  (*c*) Sandy loam produced by a thin capping of boulder clay on greensand.

(5) Brick earths giving rise to only one soil formation.

(6) Glacial giving rise to one soil formation, which, however, is not quite so uniform as the brick earth formation.

(7) Valley gravels giving rise to three soil formations:
  (*a*) A brown soil formation (referred to as "Old Brown"),
  (*b*) A heavy brown soil formation,
  (*c*) A more recent dark soil formation (referred to as "New Dark").

Samples of soil from each soil formation were then collected and submitted to chemical and mechanical analysis using the methods adopted by the Agricultural Education Association[1].

By this means, not only was it possible to verify the field observations in referring soils to distinct soil formations, but the soil formations were thus defined by a conventional method which admitted comparison of the soils with those of other parts of England and foreign countries. A glance at the results of analysis in the Appendix will show that not only has mechanical and chemical analysis differentiated between the various soil formations but an extraordinary uniformity between the samples taken from any soil formation is revealed, e.g. the greensand soils. This series of soils is differentiated from any other by the low percentage of potash and mineral salts and an almost entire absence of calcium carbonate. The coarse sand fraction is particularly high and this fact alone would be almost sufficient to distinguish it from any other series.

The uniformity of the samples collected from different parts of a soil formation is well illustrated in the case of these soils. In five soils from the brown sands the following variations were found:—

K_2O	varies between	·22–·25
P_2O_5	,,	·24–·29
$CaCO_3$	,,	·06–·09
Al_2O_3, Fe_2O_3	,,	9·34–11·87
Coarse sand	,,	51·0–59·4

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[1] *Journ. of Agric. Sci.* I. p. 470.

In the case of the dark sands we have the following variations for two samples taken:

K_2O	varies between	·13–·18
P_2O_5	,,	·11–·19
$CaCO_3$	,,	·08–·09
Fe_2O_3, Al_2O_3	,,	8·70–8·79
Coarse sand	,,	54·6–57·2

The dark sand seems to be differentiated from the brown sands by a much lower content of potash and phosphoric acid.

To take one more example, the Oxford clay soils may be considered. The Oxford clay formation has been divided into two soil formations (*a*) pure clay soils, (*b*) clay loams.

Class (*a*) is characterized by the following:

Coarse sand	14·8
Clay	29·5
$CaCO_3$	·12
K_2O	1·18
P_2O_5	·09
Fe_2O_3, Al_2O_3	15·4

Class (*b*) had the following variations in three samples analysed:

Coarse sand	varies between	20·0–23·3
Clay	,,	19·7–21·2
$CaCO_3$	,,	·06–·70
K_2O	,,	·74–·76
P_2O_5	,,	·08–·19*
Fe_2O_3, Al_2O_3	,,	10·30–11·52

The results of chemical analysis are sufficient to distinguish the Oxford clay series from all the other clay formations dealt with in this paper, for the percentage of calcium carbonate and phosphoric acid is extremely low in all the samples taken from this series.

Again mechanical analysis at once reveals the necessity for a sub-division into two soil formations, since there is a constant difference of 9 per cent. in the percentage of clay found in the two soil formations mapped from field observations.

The following table shows the average of analyses of soils on each soil formation in terms of mechanical and chemical analysis.

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* The sample containing ·19 per cent. had ·043 per cent more available P_2O_5 than any other soil in this formation and it is probable that this has been added in manures.

Definition of Soil Formations by Analysis.

	Greensand		Valley gravel			Boulder clay			Glacial	Oxford clay		Gault		Brick earths
	Brown	Dark	Old	New	Heavy	Al. wash	Pure	G. sand		Pure	Al.	Pure	Rd. ld.	
Mechanical analysis:														
Stones	6·6	8·2	11·6	9·8	5·8	7·9	4·3	5·2	5·7	1·8	1·7	7·5	2·6	2·4
Fine gravel	7·3	6·3	4·3	2·8	1·7	1·8	1·5	2·9	2·9	1·9	1·2	1·2	2·9	1·7
Coarse sand	55·2	55·9	48·3	35·5	34·1	27·5	15·9	46·8	25·5	14·8	21·8	15·3	35·1	28·3
Fine sand	12·7	13·8	18·1	19·5	21·8	18·5	14·2	18·9	20·1	9·8	16·7	12·8	17·1	18·0
Coarse silt	8·5	8·2	11·7	12·9	12·3	10·4	11·5	9·7	10·5	13·1	14·9	9·4	11·9	13·7
Fine silt	4·4	2·7	4·8	7·0	7·7	7·9	11·4	5·6	9·0	16·1	12·2	12·9	9·0	10·3
Clay	4·4	4·7	6·1	10·3	12·9	17·9	23·6	8·7	17·4	29·5	20·6	29·3	13·4	15·1
Moisture	1·26	1·29	0·9	2·0	1·0	2·6	3·7	·9	3·2	5·0	3·2	4·5	2·3	2·0
Org. matter	4·9	4·8	4·0	6·6	6·0	6·6	6·1	5·0	5·8	8·3	6·7	7·4	5·7	5·7
$CaCO_3$	·07	·09	·11	·58	·38	3·15	5·63	·08	2·7	·12	·41	3·5	·15	·80
Chemical analysis:														
Insol. residue	82·7	84·8	87·1	80·8	82·1	73·5	67·5	83·4	74·9	68·7	77·1	65·4	80·1	78·9
K_2O	·23	·15	·24	·37	45	·65	·82	·41	·68	1·18	·75	·97	·52	·51
P_2O_5	·27	·15	·22	·26	·17	·17	·15	·28	·34	·09	·13	·19	·22	·14
Fe_2O_3, Al_2O_3	10·3	8·7	7·3	8·3	9·1	11·9	13·2	9·5	10·8	15·4	10·9	15·8	10·4	9·5
CaO	·20	·20	·32	1·03	·87	2·4	4·35	·30	2·27	·65	·81	3·09	·41	1·19
MgO	·20	·14	·24	·37	·42	·44	·62	·30	·52	·67	·51	·74	·38	·43

Relationship of Soil Formations to Surface Geology.

It is of some interest to compare the soil formations with the geological formations and to ascertain the agencies which have produced varying types of soil, and maps 2 and 4 have been drawn to the same scale in order that the two sets of formations may be readily compared.

The geological map has been pieced together from numerous six inch maps which the Geological Survey Office kindly placed at my disposal. The lower half of the map has been published on the one inch scale, but the only maps obtainable for the upper half were the unpublished draft sheets at the Survey Office. Under these circumstances it is probable that the accuracy of the geological map is not very great in minor details, for this map has not been revised since the first survey was made.

In the case of boulder clay cappings resting on greensand, two small areas, marked B^1, D on the geological map 2, seem to have disappeared. It is quite probable that the thin capping of boulder clay, once existing here, has been disturbed by coprolite digging.

Again, two areas A, A^1 are marked on map 2 as belonging to the brown valley gravel soil formation but the geological map shows boulder clay and brick earths in these two spots. There appears to the present writer to be considerable evidence for mapping the geology of these areas as valley gravels. The surface features point clearly to these areas as a continuation of the main valley gravel formation marked A^2, map 2. The valley gravel formation is certainly continuous over the area A and, in the case of the area A^1, there is only a narrow depression of clay separating it from the main area A^2, map 2. The area A^1 moreover is an elevated gravel soil which appears undoubtedly at one time to have been linked up with the main area A^2. Gravels consisting of broken flints have been dug up from both the areas A and A^1 and these gravels are exactly similar to those underlying the main gravel soil formation A^2, map 2. A comparison of the following analyses reveals the similarity of the topsoil and the subsoil of the supposed valley gravel areas A and A^1 with the main valley gravel formation A^2, map 2. Analyses of brick earth and boulder clay soils in the vicinity are also given for purposes of comparison.

Not only does the analysis of the supposed valley gravel topsoil agree in every respect with that of the known valley gravel soil, but the subsoils are also identical and are widely divergent from the boulder clay and brick earth soils in the immediate vicinity. Since the geologist classifies surface geology by a consideration of the subsoil, the areas *A*, *A*[1] should be classed as valley gravels.

The area *B*, map 2, which appears on the geological map as boulder clay, seems, from field observations, to be rather different in texture from boulder clay soils. The soil is a darkish loam containing numerous small flints. A deposit of valley gravel material seems to have sufficiently altered the surface soil to justify its being classed as belonging to the valley gravel soil formation.

	Supposed valley gravels		Known valley gravels		Brick earths		Boulder clay	
	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil	Topsoil	Subsoil
Sample......	107	108	89	90	29	30	15	16
Mechanical Analysis:								
Stones	8·9	8·3	10·7	11·4	2·4	5·3	3·4	5·8
Fine gravel	3·4	3·1	3·3	3·7	1·8	2·8	1·9	1·4
Coarse sand	50·1	47·2	46·6	46·5	27·4	30·6	19·5	20·5
Fine sand	18·8	20·3	19·6	20·0	16·9	18·1	11·5	13·5
Coarse silt	10·0	10·1	11·1	12·0	11·6	11·1	9·9	—
Fine silt	5·1	5·4	5·2	5·7	11·2	8·7	10·5	—
Clay	6·6	8·9	5·7	6·7	15·4	15·5	23·5	—
Moisture	·60	·68	1·45	·95	1·82	2·42	4·11	—
Org. matter	4·27	3·00	5·12	3·25	8·75	7·40	8·83	—
$CaCO_3$	·03	—	·085	—	1·47	-	7·38	—
Chemical Analysis								
Insol residue	88·27	88·88	85·98	88·03	76·52	76·48	66·22	—
K_2O	·27	·31	·27	·31	·52	·54	·80	—
P_2O_5	·18	·15	·24	·19	·16	·14	·16	—
Al_2O_3, Fe_2O_3	6·20	6·80	6·67	6·60	9·61	10·17	13·49	—
CaO	·31	·25	·42	·36	1·78	2·24	4·84	—
MgO	·26	·27	·23	·25	·45	·50	·55	—

With the exception of these small areas indicated above, each soil series coincides in area with a geological formation.

The chief agencies producing different soil formations in the soil series are (1) river wash causing deposition of coarser grained material on the Oxford clay and boulder clay formations.

(2) Slight changes in upper and lower beds of a geological formation: e.g. in greensand the dark sands are always overlying the brown sands, and redland is held by Mr Teall[1] to be a transition bed between greensand and gault.

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[1] Dr Bonney, *Geology of Cambridgeshire.*

(3) Variations in the depth of boulder clay cappings: e.g. boulder clay on greensand is usually a sandy loam.

(4) Variation in material deposited by different river systems: e.g. old brown and new dark gravel soil formations.

PART II.

Soil Formations—their characteristic properties and agriculture.

1. OXFORD CLAY SERIES.

Dr Bonney[1] states that the Neocomian deposits of this part of Bedfordshire rest upon Oxford clay, the higher members of the Jurassic clay series being absent. Reed[2] states that the Ampthill clay is worked at Everton, but describes the lower greensand at Sandy as resting on Oxford clay. Under these circumstances it is rather a remarkable fact that soils taken from the extensive Oxford clay plain between Sandy and Tempsford should resemble Kimeridge clay soils closely in composition.

F. W. Foreman[3] describes the Kimeridge clay soils which he had collected from a number of widely separated districts as being dark brown soils, devoid of lime. He says that the dark colour distinguishes them quite readily from Oxford clay soils which are light coloured and well supplied with lime.

The present writer found that the Oxford clay soils in the district investigated were invariably very dark brown in colour and resembled the Kimeridge clay soils described by Foreman in an almost complete absence of calcium carbonate. The following are the actual percentages of calcium carbonate found by Foreman compared with the percentages found by the present writer.

	Foreman's results		*The writer's results*	
	Kimeridge clay[4] mean 2 analyses	Oxford clay mean 3 analyses	Soil from Everton	Soils from Sandy-Tempsford plain
$CaCO_3$	·18	4·07	·12	·27

Whether this plain between Sandy and Tempsford is Kimeridge or Oxford clay is a matter for the geologist and not the agriculturist to decide. It is, however, a matter of considerable importance in soil survey work, for if samples from the same geological formation show such regular variation in areas not remotely separated, additional care must be taken in interpreting the results of soil analyses as typical of the whole formation.

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[1] Dr Bonney, *Geology of Cambridgeshire.*

[2] F. R. C. Reed, *A Handbook to the Geology of Cambridgeshire.*

[3] F. W. Foreman, "Soils of Cambridgeshire." *Journ. Agric. Sci.* vol. II. pt. 2.

[4] Foreman (*loc. cit.*) describes one soil as containing 33 per cent. $CaCO_3$ but states that this soil is purely local in extent and not characteristic of Kimeridge clay.

The Oxford clay series has been divided into two soil formations.

(*a*) *The pure clay formation.*

This formation stretches out as a low-lying plain to the north of the greensand escarpment which runs through Everton and terminates at Sandy. An area of similar soil has been mapped to the east of Northill and Southill. The formation is a dark brown clay of the heaviest description. Batchelor[1] describes this soil as "a dark poor soil, coming too loose after frosts, infected by the worst of grasses, and of such general properties as to keep the cultivators poor." Owing to the slight fall to the river Ivel, the land is difficult to drain and is very wet in winter. This is particularly so of the great area to the north of Sandy and Everton. Here the poorness of the land and the low price of corn has resulted in hundreds of acres being allowed to run wild, so that now rank scrub, hawthorn, and wild rose stretch almost continuously from Everton to Tempsford. Much of this land thirty or forty years ago was in cultivation, and old inhabitants state that fair crops with plenty of straw could always be obtained. When the fall in price of wheat occurred, evidently the labour of cultivation was too high to bring a profitable return.

A few cattle are run on the pasture where it is reasonably free from scrub, but the pasture is of the very poorest, and moss seems to predominate. The land where it is cultivated grows wheat, mangel seed, horse beans, oats, clover and tares. Ploughing must always be done before Christmas or insuperable difficulties will prevent its cultivation in the Spring. The land is so wet that late Spring has arrived before it is fit to be touched, and even then the cultivator runs a good deal of risk of his newly ploughed land baking into hard intractable lumps.

The best farmers in the district all emphasize the importance of leaving one-quarter of the acreage of the farm fallow every year. The land is so infested with couch and other noxious grasses that fallowing once in four years becomes a necessity. A four-course rotation of wheat, beans, oats, fallow is usually adopted. The average yield of wheat is 28 bushels and of oats 40 bushels.

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[1] Batchelor, *Survey of Bedfordshire*, p. 12.

The Oxford clay soil to the west of Northill and Southill is rather better drained, and a slightly better type of farming is possible. Much of the land, however, is in comparatively poor pasture and woodland. Where the land is cultivated, the crops previously mentioned are grown. Usually wheat, oats and clover do fairly well, but the yield of wheat and oats is disappointing on thrashing. Mangel seed seems to be the most profitable crop for this heavy land.

Composition. Mechanical and chemical analysis reveal that this soil contains a very high percentage of the clay fraction—the highest of all the clay formations occurring in the district—and a great poverty of calcium carbonate and phosphoric acid. The potash and the iron and alumina content are both very high as one would expect in such a heavy clay soil.

The available plant food as determined by Dyer's 1 per cent. citric acid method shows a sufficiency of potash but a great deficiency in phosphoric acid. A phosphatic manure such as basic slag should be particularly valuable in the improvement of poor pasture and for all crops grown on these heavy soils.

(*b*) *Clay loam formation.*

As previously explained this formation has probably originated from an intermixture of alluvial wash with the underlying clay. It is considerably lighter in texture than the formation just considered, but it shares with it a dark brown colour and a somewhat shaley appearance. This formation is remarkably uniform throughout both areas mapped which are widely separated by the Ivel gravels.

This formation is almost entirely under cultivation, and when well manured grows good mangels, mangel seed, wheat, oats and horse beans. Brussels sprouts, late potatoes, onions, parsley and small seeds are also grown, but this formation is not very suitable for market-garden crops as the soil is somewhat heavy, and it is rather difficult of access for carting dung or produce.

Composition. This formation contains 9 per cent. less clay than the pure clay formation, with a corresponding increase in the sand fractions. The phosphoric acid is slightly higher, while the potash is a good deal lower than in the case of the pure clay formation. The phosphoric acid content at first sight appears to vary rather widely in the three samples taken from this formation. One sample, however, has been heavily manured and contains ·051 per cent. available phosphoric acid. Probably ·045 per cent. of this has been added in manures and the total content for purposes of comparison should be lowered by this percentage. The calcium carbonate content varies from ·06 to ·70 per cent., the higher figure representing the area to the south of Girtford. The area north of Sandy is low in calcium carbonate. The available plant food determinations show a sufficiency of potash but rather a low percentage of phosphoric acid, particularly in the area to the north of Sandy.

This formation is commonly manured with a light dressing of dung and a fairly large dressing of soot. Supplementary phosphatic manures such as basic slag or basic superphosphate should be valuable under these circumstances.

2. GREENSAND SERIES.

The greensand soils have been grouped into two soil formations.

(*a*) *The dark sand formation.*

This formation occurs on the escarpment of greensand between Sandy and Everton. The dark sands always seem to overlie the brown sand formation, for the dark sands are seldom more than a foot deep except on the slopes where the depth may be nearly 2 feet.

A pit on the escarpment between Sandy and Everton showed:

Grey sands	9 inches.
Carstone	1 foot.
Bright yellow sands.	

The dark sand formation is extensively covered with fir and larch plantations, but these are gradually being removed to give room for more profitable market-gardening.

Batchelor[1] describes the greensand soils as being too hilly for cultivation, and comments upon the two types of sand to be seen in the district. He says that the grey sands are invariably poorer than the brown sands and that they grow common heath (*Erica vulgaris*) and poor grasses. The present writer has also noticed this, but while Batchelor ascribes the poverty of the dark sands to a lower percentage of iron, the real reason seems to lie in a very much lower percentage of plant food.

The following figures are averages for brown and dark sands showing the percentage of iron and alumina, potash and phosphoric acid.

	Brown sands	Dark sands	Flitwick dark sand
K_2O	·23	·15	·23
P_2O_5	·27	·15	·16
Fe_2O_3, Al_2O_3	10·3	8·7	4·70

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[1] Batchelor, *Survey of Bedfordshire.*

It will be observed that the percentages of potash and phosphoric acid are very much lower in the case of the dark sands. Hall and Russell[1] found a similar low content of plant food in the uncultivated sands of the Folkestone Beds.

The dark sand formation is now extensively used for market-garden crops. The usual procedure is to grow early potatoes followed by white turnips. Sometimes early potatoes are grown as half crops with brussels sprouts or runner beans. In a few cases late potatoes are planted after taking off a crop of early potatoes. The land must be exceedingly well dunged. Thirty tons to the acre with sixty bushels of soot is commonly used on this formation.

The extensive slopes towards Sandy face the sun and are particularly early soils. These slopes are very uniform in texture and contain few stones and little gravel. Their situation, fine even texture, and depth make the soils on these slopes particularly suited to the growth of radishes and early carrots. Radishes and carrots are sown broadcast very early in February. The radishes are ready for picking in March and gradually give way to early carrots which are ready in the second week of June. Frequently parsley seed is sown among the carrots and this is ready for cutting in November and December.

It would seem, at first sight, strange that radishes are not grown on the brown sand formation. This seems to be due to the fact that the dark sand formation, particularly on the slopes just referred to, has a fine even texture and contains only a small percentage of stones and gravel. At Flitwick, another market-garden centre of Bedfordshire, the writer noticed that radishes were grown on dark greensand soils.

The following figures show the salient points of difference from the brown sand formation.

	Brown sand formation (average)	Dark sand formation (slopes facing) Sandy	Flitwick dark sand
Stones	6·6	3·9	2·3
Fine gravel	7·3	2·5	1·0
Coarse sand	55·2	66·6	68·2
Fine sand	12·7	10·5	15·5
		Radish soils	

The dark sand formation is reported by market-gardeners as being not quite so "hungry" as the brown sand formation and particularly is this so on the Sandy slopes. The custom on these slopes seems to be dung one year and soot the next.

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[1] Hall and Russell, *Agriculture and Soils of Kent, Surrey and Sussex.* Published by the Board of Agriculture and Fisheries, 1911.

Composition. The dark sand formation is characterized by very low percentages of phosphoric acid, potash, and calcium carbonate.

The soil[1] of the slopes facing Sandy is a good deal richer than the soil of the plateau above. This is due to the wash of the finer material from the plateau. The percentage of phosphoric acid in sample 47 is very high for this formation, but the available plant food is also very high and probably ·1 per cent. of phosphoric acid has been added by intense manuring.

(*b*) *The brown sand formation.*

This formation extends from Everton through Potton to Gamlingay and is almost entirely devoted to market-garden crops. Wonderful changes must have been effected since 1875 when Dr Bonney[2] described it as very sterile and as supporting little but Scotch firs. The crops grown on the brown sand formation are exactly similar to those grown on the plateau of the dark sand formation. Early potatoes are the important crop. They are followed by white turnips which grow through the Autumn and are pulled in November and December. In almost every case, the large turnips not suitable for marketing are cut up and ploughed in, and quite frequently if the crop is slightly inferior and prices are low the whole crop is turned under for green manure.

The large farmers on this formation, especially those whose farms are partly on heavy land, usually grow "full" crops of market-garden produce and are satisfied with one good crop a year They winter or keep a number of cattle, more for the sake of the manure which is made than for a profit on the cattle. They generally run a small flock of sheep which is folded on the ground with kohlrabi and summer cabbages. There is no definite system of rotation of crops on these sand lands, and particularly is this so in the case of the small market-gardeners.

The large farmers seem to arrange their crops so as to give as much rest to the land as possible between crops. Cereals are grown once in four years further to sweeten the land. The following are examples of the general procedure: brussels sprouts, late potatoes, wheat or oats, late potatoes, early potatoes, late potatoes.

Brussels sprouts and potatoes are usually dunged with 25 to 30 tons of London dung. Sometimes the dung is put on the sprouts alone and the following crop of potatoes receives a large dressing of soot.

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[1] Sample 47, Appendix.

[2] *Loc. cit.*

Composition. The brown sand formation is characterized by its high percentage of coarser particles. Thus stones and fine gravel average 13·9 per cent. while the coarse sand is 55·2 per cent. The fine silt and clay fractions are very small and the clay contains a large percentage of iron.

Chemical analysis reveals a great deficiency of calcium carbonate, a great uniformity in the percentage of potash (·22 per cent.) and a fairly high percentage of phosphoric acid. The content of calcium and magnesium salts is extremely small, but the available plant food is decidedly above the average. Probably there has been a storage of plant food due to excessive manuring, for these soils in pasture show only small quantities of available potash and phosphoric acid.

The success of the crops on this sand formation depends to a large extent on the rainfall during the period of growth. The soils are so coarse that little rise of subsoil water can take place. The problem is to introduce sufficient organic matter into the soil to conserve the water. The organic matter must be, however, of such a nature as will not open up the soil too much and let out the moisture.

3. THE GAULT SERIES.

(*a*) *Pure clay soil formation.*

This soil formation occurs as a narrow strip running between the redland soil formation and the boulder clay hills from Gamlingay to Sutton. Small areas also occur near Henlow and Stanford.

It is a particularly heavy bluish clay which is invariably wet and badly drained. This is noticeably so of the Gamlingay strip, where the drainage waters from the boulder clay hills find their way on to the surface of the gault which has been scooped out to form a long narrow depression. This soil is too heavy for market-garden crops and consequently it is used for ordinary farming purposes. It is generally farmed in conjunction with a strip of lighter land such as redland or the boulder clay on greensand formation. When drained it grows good wheat crops which, however, thrash somewhat lightly, the average yield being a little over 3 quarters per acre.

Horse beans, mangel seed, wheat and clover are the chief crops grown on the formation. Clover and sainfoin do well on this soil and give two good cuts a year.

Composition. The gault clay formation is characterized by a very high percentage of clay, 29·3 per cent., and a high percentage of calcium carbonate, 3·5 per cent. Its content of calcium carbonate distinguishes it from the Oxford clay formation which in other details resembles the gault clay formation very closely. The high percentage of clay on the other hand distinguishes it from the boulder clay soil formation which is also rich in calcium carbonate.

The potash, iron and alumina percentages are both exceedingly high while the phosphoric acid content is higher than any other pure clay soil formation in this district. Determinations of the available plant food by Dyer's method show that this soil formation has a potash content above the average, but is somewhat poor in phosphoric acid.

For spring sown crops superphosphate should be very suitable while basic slag should be an effective supplementary dressing for autumn wheat and beans, considering the wet and heavy condition of the soil.

(*b*) *The redland soil formation.*

This formation occurs as a narrow strip between the greensand and pure gault clay formations. The formation is a little over a quarter of a mile wide and runs right through from Gamlingay to Sutton.

It possesses very characteristic properties which necessitate careful handling of the soil. It must not be ploughed or cultivated while wet if it is likely to be caught in a hot sun. Should this happen it forms hard steely lumps which remain in this condition until the frosts of winter crumble them.

For this reason, the redland formation is not suitable for market-garden crops, although late potatoes and brussels sprouts are grown to some extent. Mangels and kohlrabi do well on this soil, while oats, wheat and barley are also extensively grown. A rotation of beans, wheat, roots, barley is practised by more than one farmer. This formation gives fairly good yields if suitably manured. Thus wheat averages 4–5 quarters, barley averages 5–6 quarters, mangels average 30 tons.

Composition. The texture of the redland soil formation must necessarily be somewhat uneven owing to a surface wash of greensand which grades the redland soil off into the pure brown sand formation. Sample 39 perhaps would represent the typical redland soil better than any other, but an average of the three analyses given is sufficient to give a fairly true picture of the mechanical composition and mineral constituents of this soil formation.

This formation is a coarse sandy loam deficient in calcium carbonate. The potash content ·52 per cent. and phosphoric acid ·2 per cent. lie between the percentages found in the greensand and gault soils.

The available plant food is about normal in sample 39, but is rather high in samples 13 and 19 which are both used for market-gardening.

4. GLACIAL SERIES.

There is only one soil formation of this series, which may be named the glacial gravel soil formation. There are slight differences in texture and properties in the soils occurring on this formation but, as was previously explained, these are not sufficient to demand a subdivision.

For convenience in describing the formation it may be divided into two groups. (*a*) The Blunham glacial gravel area almost entirely devoted to market-garden crops; (*b*) isolated areas occurring as thin cappings on boulder clay, generally held by large farmers in conjunction with heavier land.

The Blunham area is of considerable extent. The soil is heavier in texture than any of the valley gravel formations and contains fewer stones, which are generally smooth with flat surfaces. The soil is a cool good working loam very suitable for peas, runner beans, brussels sprouts, late potatoes, onions, marrows and small seeds. The soil is not cropped quite so heavily as the valley gravel soil formations. The owner usually has to be satisfied with one crop a year but in some cases early peas are followed by brussels sprouts, or failing this, by spring cabbages. The soil is, as a rule, not so heavily manured as the greensand or valley gravel soil formations, the land being dunged only once in two years.

In the case of the smaller areas of glacial gravel, occupied by larger farmers, one finds that they are following the market-gardening practice, either sub-letting their best fields to the market-gardener, or using them themselves for market-garden crops.

Late potatoes, brussels sprouts, onions, parsley and strawberries all flourish exceedingly on these rich glacial areas, while cereal crops and roots give very large yields. Potatoes with very indifferent treatment average 8 tons; onions average 8–12 tons, depending on the season. Very high yields of wheat are frequently obtainable, as high as 50 bushels to the acre being the yield in some years. The soil is so good that three cereal crops are often taken consecutively, each yielding very well. Wheat would average out at about 5 quarters and oats 10 quarters per acre. Strawberries too succeed particularly well and frequently give over 2 tons of fruit per acre.

Composition. The glacial soils are loams containing 8·5–9 per cent. stones and fine gravel, 40–50 per cent. sand fractions, 15–19 per cent. clay fraction. They are well supplied with calcium carbonate and potash. The phosphoric acid in sample 53, from one of the small areas, is very high ·47 per cent., while the available phosphate is outstandingly so for a soil which is not very heavily manured. The sample is somewhat low in available potash and probably would benefit by dressings of potash manures, particularly for market-garden crops. Sample 51 from the Blunham district contains available plant food to an extent only slightly above normal.

5. BRICK EARTHS.

The material of this formation, laid down by rivers of an early period, is extremely uniform throughout the district, and therefore constitutes only one soil formation. The topsoil is a brown heavy loam, resembling the glacial soil formation very closely in texture, but containing fewer stones and gravel. The subsoil to a depth of several feet is characterized by a large percentage of rounded pieces of chalk, together with some fine gravel. The soil is used extensively for such crops as late potatoes, brussels sprouts, onions, parsnips, mangels and to some extent cereals. Only one crop a year is taken off this soil formation and consequently the land is not very heavily manured: 25–30 tons of dung once in three years seems to be the usual dressing.

Brussels sprouts or late potatoes usually receive the manure, while the following crops, onions and parsnips, are often dressed with soot. Malt dust is frequently used for onions on this formation, and is held in high esteem. There is no definite system of rotation of crops on this soil formation, but care is taken not to put the same crop on the land too frequently.

Composition. The brick earth soil formation is probably derived from material re-sorted from boulder clay. This being so, one would naturally expect a rather high content of calcium carbonate and potash and a somewhat low percentage of phosphoric acid. Chemical analysis reveals this in a marked manner, the only exception being the low percentage of calcium carbonate in the topsoil, sample 23. All other figures in the chemical analyses are strikingly uniform in both samples. The available plant food is about normal for the soils on this formation. Sample 29 which has till recently been in woodland contains rather a specially high percentage of nitrogen and available phosphoric acid.

6. BOULDER CLAY SERIES.

(*a*) *Pure boulder clay soil formation.*

This formation is characteristic of much of the higher ground in the district which is largely occupied by pasture, woodland, and mixed farming. In some places the pasture is extremely poor and is slowly going back into scrub. This land has at some period been quite largely used for cereal crops, for numerous fields can be seen laid in baulks. It is a fairly heavy clay soil but the high percentage of chalk, stones and fine gravel, added to the fact of better natural drainage, makes its cultivation easier than the pure gault or Oxford clay soil formations. The arable land of this soil formation is used for cereals, horse beans, mangels, mangel seed and clover. Brussels sprouts can be grown of good quality, and in places where the soil is a little deeper late potatoes and onions do moderately well.

The usual treatment of the land is somewhat as follows. Horse beans (15 tons dung), wheat, oats without manure, potatoes (if the soil is deep enough) (20 tons dung). Sometimes soot is used alone for potatoes without dung. Needless to say the crop in these cases is not very large. Clover seed is introduced occasionally into the above rotation. Clover, sainfoin and lucerne, where grown, do well on this soil formation, but cereal crops thrash rather lightly.

Composition. This formation is characterized by the rather high but constant content of clay, 23·6 per cent., a high content of calcium carbonate, 4·8 per cent. (average) and a low content of phosphoric acid, ·15 per cent. (average). The available potash is usually about normal but the phosphoric acid is invariably extremely low. One sample taken from small-holding land but recently acquired by a parish council for small market-gardeners contained an abnormally low content, ·001 per cent. of available phosphoric acid.

Sample 37 is an example of a deeper soil, resulting from local drift, which is very heavily manured for potatoes, onions and parsnips.

The pure boulder clay soil formation is one which requires scientific treatment. Basic slag has wrought wonders at Croydon in the improvement of poor pasture, and phosphatic dressings are needed on this formation for nearly every crop.

(*b*) *Clay loam formation* (*alluvial on boulder clay*).

This formation occupies a small area on the right-hand side of the Ivel near Langford. It possesses a texture considerably lighter than the pure boulder clay soil formation and is therefore used extensively for market-garden crops. Onions, parsley, parsnips, brussels sprouts and late potatoes are the principal market-garden crops, but mangel seed, horse beans, cereals and clover are also grown.

The treatment adopted by the market-gardeners for this soil is very similar to that in practice on the brick earths soil formation. Twenty tons of dung are used for potatoes or brussels sprouts and during the following two years soot and malt dust are used for parsnips and onions. The average yield of crops is very similar to the yields on the brick earths soil formation.

Composition. This formation contains a higher percentage of stones and coarser particles than the pure clay formation just considered. The soil is well supplied with calcium carbonate and the available plant food, particularly phosphates, is very much higher than that of the pure boulder clay soils.

(*c*) *Sandy loam soil formation* (*boulder clay on greensand*).

This formation occurs in the district only in two small areas but its characteristics are so different from true boulder clay that it must be designated as a distinct soil formation. This formation only occurs where the boulder clay covering the greensand forms a thin capping which intermixed with the sand results in a loamy soil. The texture of this soil formation varies even on the small areas mapped, owing to varying thickness in the capping of boulder clay. It resembles the redland soil formation in its characteristics and possesses the same property of going steely if caught wet with a hot sun.

This soil formation is mainly used for mixed farming but a small area of market-garden crops is grown. Brussels sprouts and late potatoes are the most important of these crops. Mangels, kohlrabi, barley and oats all do fairly well on this soil formation.

Composition. The only sample taken was from the more sandy part of this formation, since it was only on the more sandy parts that market-garden crops were grown. The sample is obviously a mixture of boulder clay and greensand and its properties are intermediate. The calcium carbonate is, however, very low, resembling the brown sand soil formation closely in this respect.

7. VALLEY GRAVEL SERIES.

(*a*) *Old brown soil formation.*

This soil formation derives its name from the extensive valley gravel deposits at higher levels on either side of the Ivel, in the neighbourhood of Biggleswade and Stanford. These gravels were evidently laid down by a much larger river which probably flowed through greensand country from Flitwick to Shefford. The characteristic colour of these deposits, especially the extensive area from Broom to Stanford, is brown, hence the name "old brown" was applied to it to distinguish it from the more recent dark soil formation at a lower level.

It was found that the remaining gravel soils of the district, with the exception of a small area, which will be described later, could be referred to either one or the other of these two soil formations. It was found, however, convenient when giving the analyses of the gravel soils in the Appendix to group the samples together according to the localities from which they were taken. Thus any small variation due to local causes would be revealed. The area which has been referred to as an exception will be discussed later under the heading "Heavy brown soil formation."

The old brown soil formation is of such great extent and its areas are so widely separated that it is only to be expected that local causes will produce some slight variations in texture in different localities. There is, however, such a great resemblance in general characteristics that all the areas have been included as one soil formation. In order, however, to describe fully its properties and the effect of local conditions it has been subdivided into the following areas:

(1) Biggleswade Plateau.
(2) Stanford-Broom Plateau.
(3) Biggleswade Common Plain.
(4) Ouse Brown Gravels.

The Biggleswade plateau on the east side of the London Road resembles the Stanford-Broom plateau very closely in mineral structure and texture, but it has been greatly improved by heavy manuring with London dung. There is easy access to the Biggleswade railway station and evidently it has been under market-garden culture for a considerable number of years. The organic matter has been very notably increased by this heavy manuring and this makes it far more suitable for the growth of such crops as carrots and parsnips than it otherwise would be.

The Stanford-Broom plateau has been taken up by market-gardeners only in comparatively recent years and, owing to the distance from any railway station, the quantity of dung applied is very much less than in the previous case. It also has the disadvantage of a slightly increased percentage of stones and fine gravel, in one sample these fractions being 10 per cent. higher than the average for the Biggleswade soils. Owing to the small percentage of organic matter the soil on the Stanford-

Broom plateau is apt to form a thin hard cake on the surface. This makes the soil very unsuitable for such crops as carrots and parsnips, because it would be impossible to get a good "stand" of seedlings.

The Biggleswade Common plain and the Ouse gravels resemble the Stanford-Broom plateau very closely and in both cases the texture is not so good as on the Biggleswade plateau. The soil on the Biggleswade Common plain is rather more uniform in texture than the Stanford-Broom plateau. This is due to a wash of greensand material from the neighbouring escarpment. The subsoil shows just as much stones as the Biggleswade plateau soils, showing that the wash is purely a surface one. The agricultural treatment of this area is precisely similar to that of the Stanford-Broom plateau and mechanical or chemical analysis reveals an almost identical composition. The Ouse gravel soils are very similar in colour and properties to the soils of the Stanford-Broom plateau. In places, however, the underlying gravel beds come very near to the surface and make market-gardening a precarious occupation in dry seasons. Sample 65 is an instance of this, but sample 57 perhaps more adequately represents the Ouse gravel soils. This sample contains only 10 per cent. of stones which is very similar to the average content of the old brown soil formation.

The old brown soil formation taken as a whole is associated with the following market-garden crops: late potatoes, brussels sprouts, early potatoes, green peas and spring cabbages. Smaller quantities of runner beans, onions, parsley and parsnips are also grown on this formation. A considerable acreage of the formation is farmed, mangel seed, cereals and clover being the principal crops.

The large owners on this formation are gradually introducing market-garden crops in place of ordinary farm crops. They invariably winter a number of cattle in order to make farmyard manure for the hungry soil. In many cases little profit is made on the animals, but a supply of farmyard manure on the land even outweighs a small deficit on the animals.

As a rule these farmers aim at having one big crop a year rather than two small ones. The land is hardly in good enough "heart" to grow more than one good crop a year, but early potatoes followed by brussels sprouts is not uncommon. Early peas followed by spring cabbages and then an autumn crop, making three crops in two years, are also seen. The following example illustrates the rotation usually adopted by the larger owners on this soil formation: Potatoes (manured 20 tons farmyard manure). Brussels sprouts (manured 1 cwt. nitrate of soda and 1 cwt. common salt). Oats without manure. Clover and rye or wheat. The average yield is 36 bushels of wheat and 5–6 quarters of oats to the acre.

The smaller market-gardeners on the Stanford-Broom plateau find it very difficult to obtain London dung and consequently rely greatly on soot, with poor results. Artificial manures are coming slowly into use, owing to this difficulty in obtaining dung, and one or two market-gardeners using them, it is true without much science, are nevertheless obtaining larger crops than their neighbours. The smaller market-gardeners appear to have no definite system of rotation of crops. They grow potatoes and brussels sprouts with an occasional crop of peas and cereals.

There is considerable rivalry between the Biggleswade and Potton market-gardeners in putting their crops first on the market. The Potton market-gardeners on the brown sand formation usually manage to place early potatoes on the market almost a fortnight ahead of the Biggleswade gardeners. This is due to a slightly heavier soil, in the case of the Biggleswade plateau, which retains the water to such an extent as to make this difference in earliness between the two soils.

The following analyses show the water content of the Biggleswade plateau and the brown sand formation at two different periods of the year. The samples were taken from soils on the same plateau at about corresponding altitudes.

	Depth of Sample	Greensand brown sand formation	Old brown soil formation (Biggleswade plateau)
Sampled January, 1913.	0 –9″	15·0 per cent. water	16·9 per cent. water
	9″–18″	12·8	12·9
	18″–27″	10·6	12·4
Sampled April 24th, 1913.	0 –9″	12·0 per cent. water	14·8 per cent. water
	9″–18″	11·2	12·9
	18″–27″	9·1	12·1

Composition. Mechanical and chemical analysis reveals a striking uniformity throughout all the samples of the old brown soil formation. They are all coarse gravel sands containing rather less coarse sand and more clay than the greensand brown sand formation. The content of potash and phosphoric acid is very similar to that found in the brown greensand soil formation. The old brown formation is largely derived from greensand material and this accounts for the close resemblance in the percentages of these ingredients. The soils of this formation are very deficient in calcium carbonate. The available phosphoric is usually high, but the percentage of available potash tends to be low, particularly on the Stanford-Broom plateau. Sample 65 from the Ouse brown gravels contains very high percentages of available potash and phosphoric acid. This has probably largely been added in manures, for if the available content in samples 57 and 65 be subtracted from the total content of these ingredients, identical percentages for phosphoric acid and potash are obtained in both cases.

(*b*) *Heavy brown soil formation.*

This formation was laid down about the same time as the old brown formation, but differs from it in containing nearly 10 per cent. more of the two finer fractions, clay and fine silt. It is situated on the western edge of the old brown formation and runs from a little north of Southill to Upper Caldecote.

The crops associated with this formation are early potatoes, late potatoes, peas, spring cabbages, mangel seed, onions, cauliflowers, brussels sprouts and cereals. Mangel seed, peas and spring cabbages seem to be particularly suited to this soil. The farming practice on this formation is very similar to that used on the old brown formation and therefore need not be repeated.

Composition. Chemical analysis shows that this soil formation contains a much higher percentage of mineral salts and calcium carbonate than the old brown formation. The only exception to this is phosphoric acid which is rather low.

(*c*) *New dark soil formation.*

This soil formation is characterized by its dark colour and a more loamy texture than the old brown soil formation. It is more recent in age and the underlying gravels do not come so near to the surface. This formation has probably resulted from the mixture of material brought down by the tributaries of the Ivel, which join the main stream in the vicinity of Langford. The following analyses show the composition of sludges taken from the river bed of the Hiz and the main river.

	(*a*) Clifton-Shefford Main Stream	(*b*) Hiz
Moisture	·97 per cent.	2·57 per cent.
Org. matter	4·15	19·2
Nitrogen	·136	·84
Calcium carb.	3·25	34·6
Insol. residue	85·96	32·81
K_2O	·17	·50
P_2O_5	·18	·74
Fe_2O_3, Al_2O_3	5·46	9·67
CaO	2·04	18·95
MgO	·17	·74

The Clifton-Shefford branch is the main stream which flows continuously over greensand country from above Flitwick to Shefford. The sludge from this stream consists to a large extent of fine sand and coarse silt particles. It is comparatively low in calcium carbonate, potash, organic matter and all mineral salts. The Hiz tributary which at one time must have been very much larger flows over gault and chalk formations. The sludge from this stream is extraordinarily rich in calcium carbonate, organic matter, potash and phosphates. It contains a large percentage of the finer fractions such as clay and fine silt. Evidently it was the mixture of the material of these two streams which has produced the fine textured and fertile soils of the new dark soil formation. This soil formation is entirely devoted to market-gardening and probably this is the soil which was so famous for onions early in the nineteenth century. The soil in the neighbourhood of Biggleswade and Sandy has been devoted to market-gardening for centuries[1].

The soil on this formation is a fairly cool light loam with sufficient of the finer particles to prevent it from drying out in hot seasons. It is, however, underlain by gravel, at some depth below the surface and consequently the soil is well drained. Brussels sprouts, early and late potatoes, onions, carrots, parsnips, spring cabbages, parsley, peas and runner beans are all grown on this soil formation. It is the custom on this soil to grow three crops in two years. Early potatoes are frequently grown as a half crop with brussels sprouts or runner beans. Early peas are sometimes followed by brussels sprouts, but more frequently by spring cabbages. A main crop will usually follow on the land during the next year. Parsley is always associated with onions or carrots and is never grown as a separate crop.

The new dark soil formation is the most productive of all the soils used for market-garden crops. The land is extremely well manured with London dung and soot, but at present the quantity of artificial manure used is very small, with the exception of a top dressing of 1 cwt. nitrate of soda and 1 cwt. of common salt for forcing crops.

The crops on this soil formation are invariably much better in appearance and yield than those on the old brown soil formation. The following would be the average yields of the chief crops in fairly good seasons: Parsnips 16–20 tons, onions 10–14 tons, potatoes 8–10 tons, early potatoes (half crop) 3½–5 tons.

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[1] Batchelor, *loc. cit.*

Composition. The texture of this formation is rather more sandy in the neighbourhood of Langford and Eynesbury, but otherwise is comparatively uniform over its whole extent. The soil is usually well supplied with calcium carbonate, while the percentage of organic matter and potash is invariably higher than that of the old brown formation. The available plant food is very high on all the soils which have been used for market-garden culture, but even soils in pasture show a high content of available phosphoric acid (·059 per cent.).

PART III.

Relation of Crops to Soil Formations.

Hall and Russell[1] have demonstrated that by plotting the parish returns of a crop by means of dots on the areas of the various soils of each parish it was possible to bring out certain relationships between crop and soil, when the crop map thus constructed was compared with the geological map of the same area. Each dot represented a certain number of acres of some crop and the density of dots on the crop map revealed areas which were adapted to the growth of this crop. Thus they were able to show that hops and potatoes were each associated with certain types of soil.

This method appears to meet with considerable success where the soil occurring in a number of adjoining parishes is uniform over the whole area, but where a number of soil formations are to be found in one parish or adjoining parishes it is obvious that little relationship can be shown to exist by indiscriminate application of this method. Thus the relationship might not be brought out if the parish returns for a crop were to be dotted evenly over the whole parish area, irrespective of the fact that the crop may only occur on one of the several soil formations.

In the market-garden district of Biggleswade, the writer found many soil formations with distinct characteristics occurring in one parish. Any method of plotting the acreage of a crop indiscriminately over the parish area would thus have been useless for showing the real distribution of crops. Two maps, Nos. 5 and 6, have been constructed to show the result obtained by this method. Maps Nos. 7 and 8 have been made by dotting the acreage of the same crops over the area of the soil formation on which they occur. A comparison of the two sets of maps will reveal a great difference in the effect produced by the two methods of construction.

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[1] Hall and Russell, *Agriculture and Soils of Kent, Surrey and Sussex.* Published by the Board of Agriculture and Fisheries, 1911.

The details of the method used by the writer were as follows: The acreage of each crop on every soil formation was measured up. The acreage, choosing some suitable unit, was then dotted on the areas of a soil formation, which carried that crop. The 6 inch Ordnance Survey maps, which show the fields, were used in obtaining crop statistics. The crops on each field were measured up and the total acreage of each crop was worked out for each soil formation on which it was found to be growing on every sheet of the 6 inch map. The figures were then plotted separately for each 6 inch sheet on an outline map of the soil formations reduced to a one inch scale, and it was then found that certain soil formations were markedly associated with some particular crop.

The construction of these maps has involved a tremendous amount of labour, for the acreage of every crop on an area of nearly 100 square miles had to be estimated and then tabulated under the various soil formations with which they were associated. When it is borne in mind that the market-gardener grows a variety of crops on a small acreage of ground, the labour involved in making crop returns and calculating acreages will easily be seen to be no small one. Reproductions of the crop maps which show the distribution of twenty-one crops over the various soil formations are shown in the Appendix. An inspection of these maps at once reveals soils which farmers have found by experience to be well suited to the growth of various market-garden crops.

In the following short summary of the relationships brought out by these maps between crop and soil it is proposed only to indicate those which are most striking. The presence of smaller quantities of a crop on any formation will not be mentioned, as these have already been dealt with in a general way when describing the soil formations. The following are the most striking relationships:

Map 9. White turnips associated with brown and dark greensand formations.

Map 10. Early potatoes associated with brown and dark greensand and valley gravel soil formations.

Map 11. Carrots associated with dark and brown greensand soils, and the new dark gravel soil formation. The brown greensand grows more late carrots than early ones.

Map 12. Onions; new dark gravel soil formation, wash on Oxford clay, wash on boulder clay, and glacial soil formations.

Map 13. Runner beans; dark greensand soil formation, glacial and new dark gravel soil formations.

Map 14. Parsley; glacial, new dark gravel, dark greensand and parts of the old brown gravel soil formations.

Map 15. Late potatoes; grown on every soil formation with the exception of the pure Oxford and gault clay soil formations. They are particularly associated with the old brown gravel, glacial, boulder clay with wash and brick earth soil formations.

Map 16. Brussels sprouts; very wide distribution. They are grown on all classes of soil with the exception of the pure Oxford and gault clay soil formations. They are best suited to the loams and heavy loams such as the new dark gravel, glacial, boulder clay with wash and brick earth soil formations. Brussels sprouts are not suited to the light soils which in this district are all deficient in calcium carbonate and consequently get very "sick" if this crop is grown at all frequently. They are apt to "break away" during a mild winter on the light soils and the quality of sprouts is not nearly so good as that on the heavier soil formations.

Map 17. Parsnips; very similar in distribution to onions. The new dark gravel and the boulder clay with wash soil formations are the best soils for this crop.

Map 18. Marrows; not very extensively grown but closely associated with the new dark gravel and glacial soil formations.

Map 19. Green peas; glacial and all valley gravel soil formations.

Map 20. Spring cabbages; new dark gravel, brick earth and heavy brown gravel soil formations.

Map 21. Cauliflowers; very little grown but associated with the old brown and heavy brown gravel soil formations.

Map 22. Mangel seed; heavier types of soil such as pure boulder clay, wash on Oxford clay, wash on boulder clay, glacial and heavy brown gravel soil formations.

Map 6. Horse beans; heaviest types of soil such as pure Oxford clay, pure boulder clay, pure gault clay soil formations.

Map 23. Roots; loams and heavy loams such as wash on Oxford clay and particularly with redland and boulder clay on greensand soil formations. (Mangels, swedes and kohlrabi are included under the name of roots.)

Map 24. Small seeds (onion, carrot, parsley, turnip seed); glacial, Oxford clay with wash and brick earth soil formations.

Map 25. Legumes (clover, sainfoin, lucerne); heavier types of soil, but also with the old brown gravel soil formation.

Map 26. Cereals; wide distribution, but heavier soils more closely associated with them than other soil formations.

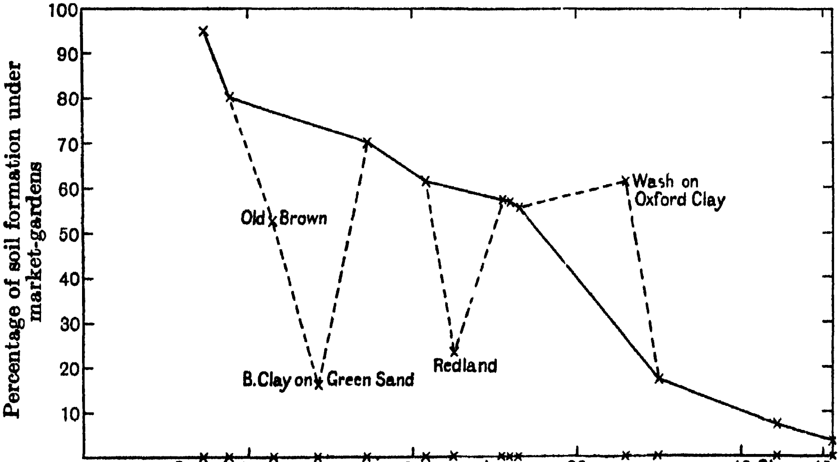
Map 8. Pasture; alluvial, pure Oxford clay and pure boulder clay soil formations.

The following table has been prepared to show the extent to which the various soil formations are used for market-gardening. The percentages given are the percentages of arable land on each soil formation, occupied by (1) market-garden crops as a whole, (2) farm crops. For this purpose brussels sprouts and late potatoes have been considered as market-garden crops.

Series	Soil formation	Market-garden crops	Farm crops
Oxford clay	Pure	4·0	96·0
„	Clay with wash	61·3	38·7
Greensand	Brown sands	80·0	20·0
„	Dark sands	94·7	5·3
Gault	Pure	7·8	92·2
„	Redland	23·6	76·4
Boulder clay	Pure	17·4	82·6
„	With wash	57·0	43·1
„	On greensand	16·3	83·7
Brick earths	Brick earths	57·5	42·5
Valley gravels	Old brown	52·7	47·3
„	New dark	70·4	29·6
„	Heavy brown	61·7	38·3
Glacial	Glacial	55·6	44·4

Below a graph has been drawn to show the relationship of market-gardening, as a whole, to the texture of the various soil formations. For this purpose the percentage of arable land devoted to market-gardening, on each soil formation, has been plotted against the percentage of clay and fine silt, together, in the soils of each formation. It will be noticed that the area devoted to market-gardening decreases fairly regularly with an increasing percentage of the finer particles. There are four exceptions which are shown by dotted lines on the graph. The redland and the boulder clay on greensand soil formations fall considerably below the main line. This is due to the fact that both these soil formations are difficult to cultivate owing to their property of baking to steely lumps if caught by a hot sun. Both soil formations are rather small in area and perhaps it is hardly fair to include them in the diagram at all. The old brown gravel soil formation also falls below the main line curve, but, as previously explained, this formation is farmed to a large extent owing to the great difficulty in obtaining London dung so far away from any railway station.

The one exception to come above the main line is that of the Oxford clay with wash soil formation. This formation has been divided into allotments on account of the great demand for small holdings. The soil is not very suitable for most market-garden crops, but no other land is available for market-garden purposes.



Texture (clay + fine silt)

The following table shows at a glance the proportion of each market-garden crop on each soil formation. The percentages given are percentages of the total arable land on each soil formation. These figures are not tabulated for the purpose of finding the soil formation most suited to a crop, because some of the soil formations are so small in area that it would hardly be safe to do this. The suitability of a crop to a soil formation is best divined by a study of the crop maps. This table does, however, bring out the various proportions of the different crops grown on any soil formation.

Table showing proportions of the various market-garden crops grown on each soil.

Figures give percentages of the total area occupied by each crop.

Crops	Oxford clay		Greensand		Gault		Boulder clay			Brick earth soils	Glacial soils	Valley gravels		
	Pure	With wash	Brown sands	Dark sands	Pure	Red-land	Pure	With wash	On green-sand			Old brown	Heavy brown	New dark
White turnips	—	—	23·3	32·3	—	3·4	—	—	2·3	—	—	·3	—	—
Cereals	72·5	25·0	14·2	5·3	63·5	60·0	62·5	31·9	62·3	40·0	36·5	35·2	26·2	22·5
Roots	—	4·0	4·8	—	5·9	9·7	1·9	2·5	11·4	·4	2·1	2·1	—	1·5
Brussels sprouts	1·9	23·5	18·8	16·0	4·9	11·0	8·3	21·3	2·5	16·9	19·8	20·5	6·3	21·2
Parsley	—	3·8	1·5	5·8	—	—	·5	1·5	—	2·1	7·8	3·4	3·4	5·5
Legumes	14·3	6·3	·9	—	14·4	6·7	10·3	4·7	8·4	1·3	4·2	6·7	—	2·3
Late potatoes	2·2	11·4	12·1	17·6	2·4	4·3	5·5	16·3	10·2	20·7	14·2	13·9	19·4	11·7
Early potatoes	—	2·8	61·0	66·5	—	·6	—	—	—	—	6·8	12·2	25·8	21·0
Cauliflowers	—	—	—	—	—	·8	—	—	—	—	—	1·5	8·7	·5
Summer cabbages	—	—	·2	—	—	·8	—	—	—	—	—	·9	—	1·2
Carrots	—	1·0	16·2	9·8	—	—	—	2·3	—	1·3	·8	1·1	—	8·3
Onions	—	7·7	·7	—	—	—	1·2	6·7	—	3·8	6·8	3·6	8·2	6·7
Runner beans	—	1·6	5·3	15·4	—	—	—	—	—	—	6·0	1·7	—	3·2
Beet	—	—	1·3	—	—	—	—	·6	—	—	—	—	—	·6
Parsnips	—	1·8	·8	—	—	—	·2	6·9	1·3	2·5	·5	3·1	2·6	5·2
Marrows	—	·7	·3	2·5	—	—	—	—	—	—	2·8	·7	2·3	2·0
Mangel seed	1·5	2·8	—	—	1·3	—	4·0	3·3	—	—	3·1	2·6	12·1	2·4
Horse beans	7·0	·5	—	—	5·3	—	3·9	·7	1·3	·8	1·6	·7	—	·9
Peas	—	1·6	·4	—	—	—	·3	—	—	—	4·0	5·9	16·8	2·9
Spring cabbages	—	1·7	4·8	·7	—	·6	—	·8	—	5·5	1·6	3·6	11·3	6·5
Small seed	—	2·4	—	—	·5	—	·5	—	—	2·2	3·6	1·3	—	·7
Asparagus	—	—	—	—	—	—	—	—	—	—	—	·3	—	1·2

SUMMARY.

The writer has demonstrated that the geological formations in this district give rise to a series of related soils which exhibit this relationship more closely in the mineral constituents than in the texture.

This series of soils may be usually divided into two or more soil formations having distinct characteristics which require different agricultural treatment. Mechanical and chemical analyses have been shown to define the soil formations very clearly, revealing striking uniformity among samples taken from the same soil formation and marking it off definitely from any other.

The writer has found the geology of the district to differ in several points from the unpublished draft sheets of the Geological Survey Department. Reasons have been given for any change on the geological map which the writer thinks desirable.

The soil formations have been described and their agricultural treatment correlated with the results of mechanical and chemical analyses.

Crop maps have been constructed showing the distribution of crops over the various soil formations and the relationships between crop and soil thus occurring have been described. The extent to which each soil formation is used for market-garden culture has also been shown.

The failure of the method of plotting the acreage of a crop indiscriminately over the parish area, in the Biggleswade market-garden district, has been demonstrated.

In all county soil surveys where there are extensive "quarternary" deposits, giving rise to variations in the soils overlying the geological formations, or where numerous geological formations outcrop within a small area, the writer would suggest that in addition to the ordinary soil survey usually conducted one or two small areas of about 30 square miles in country of typical variation should be minutely investigated in the manner described above in the present paper. If this is done, the author feels sure that many characteristics of soil and crop relationships will be brought out which otherwise would escape notice.

In conclusion, I have to thank Professor Wood for suggesting the subject for research and for his helpful advice during the progress of the work, and I wish also to thank Mr L. F. Newman who kindly placed at my disposal his long experience of soil survey work.

APPENDIX.

Oxford Clay Soils.

	Pure clay formation		Clay with wash			
Sample ...	69	70	55	63	64	67
Stones	1·8	1·0	1·5	2·2	2·7	1·4
Mechanical analysis:		subsoil			subsoil	
Fine gravel	1·9	1·8	1·1	1·7	1·0	·9
Coarse sand	14·8	13·7	23·3	20·0	21·0	21·7
Fine sand	9·8	8·4	15·7	16·4	15·1	18·0
Coarse silt	13·1	—	15·8	13·7	—	15·3
Fine silt	16·1	—	11·4	12·1	—	13·2
Clay	29·5	—	20·8	21·2	—	19·7
Moisture	5·01	—	3·1	3·5	—	2·9
Org. matter	8·29	—	6·1	8·4	—	5·6
$CaCO_3$	·12	—	·47	·70	—	·06
Total	98·6	—	97·8	97·7	—	97·3
Chemical analysis:						
Moisture	5·01	—	3·07	3·54	—	2·91
Org. matter	8·29	—	6·09	8·44	—	5·59
Nitrogen	·186	—	·182	·250	—	·152
Insol. residue	68·71	—	77·88	73·59	—	79·74
K_2O	1·18	—	·75	·76	—	·74
P_2O_5	·09	—	·12	·19	—	·08
Al_2O_3, Fe_2O_3	15·40	—	11·05	11·52	—	10·30
CaO	·65	—	·77	1·14	—	·51
MgO	·67	—	·47	·69	—	·46
Available 1 % citric acid:						
K_2O	·025	—	·031	·028	—	·021
P_2O_5	·002	—	·009	·051	—	·008

Greensand Soils.

	Brown sands						Dark sands			
Sample ...	1	2	33	59	61	97	47*	71	77	99†
Stones	5·3	7·4	7·0	10·5	3·5	4·7	3·9	6·5	9·9	2·3
Mechanical analysis:		subsoil								
Fine gravel	11·3	8·8	3·6	9 8	4·5	—	2·5	7·6	5·0	1·0
Coarse sand	56·1	57·8	54·3	51·0	59·4	—	66·6	54·6	57·2	68·2
Fine sand	10·5	12·1	14·9	13·7	11·8	—	10·5	14·3	13·4	15·5
Coarse silt	7·5	6·3	8·3	9·9	8 4	—	4 3	9·8	6·6	3·1
Fine silt	3·5	4·1	5·8	3 4	5·0	—	3·9	1·7	3·6	3·1
Clay	4·7	5·7	6·4	2·9	3·5	—	4·0	3·9	5·4	3·7
Moisture	1·23	1·3	·90	1·8	1·2	—	1·4	1·4	1·2	·5
Org. matter	3·76	2·9	4·48	5·9	5 6	—	4·8	4·7	5·0	3·9
$CaCO_3$	·06	—	·07	·07	·09	·065	·10	·08	·09	·35
Total	98·65	99·0	98·7	98·5	99·5	—	98·1	98·1	97·5	99·35
Chemical analysis:										
Moisture	1·23	1·30	·90	1·77	1·16	·83	1·35	1·42	1·15	·55
Org. matter	3 76	2·90	4·48	5·92	5·57	7·87	4·82	4·68	5·00	3·90
Nitrogen	·101	·064	·127	·145	·162	·220	·145	·106	·122	·120
Insol. residue	83·70	83·70	82·72	81·15	83·05	80·37	85·74	85·11	84·47	89·94
K_2O	·22	·24	·22	·25	·22	·22	·24	·13	·18	·23
P_2O_5	·27	·25	·26	·24	·29	·28	·43	·11	·19	·16
Al_2O_3, Fe_2O_3	10·71	11·38	11·05	10·28	9·34	11·87	7·02	8·70	8·79	4·70
CaO	·10	·10	·25	·24	·23	·19	·53	·08	·33	·32
MgO	·16	·16	·25	·19	·19	·21	·21	·12	·16	·14
Fe_2O_3	8·56	9·00	—	—	—	—	—	—	—	—
Available 1 % citric acid:										
K_2O	·017	—	·023	·058	—	·017	·022	·014	·019	·015
P_2O_5	·034	—	·036	·061	—	·011	·084	·012	·037	·042

* Soil from Sandy slopes.

† Soil from Flitwick.

Gault Clay Soils.

	Pure clay formation		Redland formation			
Sample ...	41	42	13	14	19	39
Stones	7·5	1·1	2·3	1·8	3·5	2·0
Mechanical analysis:		subsoil		subsoil		
Fine gravel	1·2	1·5	2·2	2·2	3·9	2·5
Coarse sand	15·3	13·4	29·9	24·2	40·9	34·4
Fine sand	12·8	11·4	12·6	12·2	15·4	23·3
Coarse silt	9·4	—	12·6	—	11·2	12·1
Fine silt	12·9	—	10·4	—	8·0	8·5
Clay	29·3	—	18·4	—	9·9	12·0
Moisture	4·50	—	3·20	—	2·24	1·50
Org. matter	7·80	—	6·74	—	6·04	4·36
$CaCO_3$	3·52	—	·09	—	·29	·05
Total	96·7	—	96·1	—	97·9	98·7
Chemical analysis:						
Moisture	4·50	—	3·20	—	2·24	1·50
Org. matter	7·80	—	6·74	—	6·04	4·36
Nitrogen	·217	—	·185	—	·168	·129
Insol. residue	65·40	—	76·33	—	81·02	82·96
K_2O	·97	—	·65	—	·43	·47
P_2O_5	·19	—	·21	—	·29	·16
Al_2O_3, Fe_2O_3	15·83	—	11·70	—	9·46	10·13
CaO	3·09	—	·52	—	·36	·35
MgO	·74	—	·50	—	·27	·36
Available 1 % citric acid:						
K_2O	·031	—	·026	—	·018	·022
P_2O_5	·014	—	·048	—	·061	·027

Boulder Clay Soils.

	Pure clay soil formation					
ıple ...	8	15	16	43	6	37
	4·8	3·4	5·8	4·7	—	2·9
analysis:			subsoil			
ravel	·7	1·9	1·4	1·8	—	1·2
sand	11·6	19·5	20·5	16·6	—	20·6
and	14·6	11·5	13·5	16·6	—	13·0
silt	10·7	9·9	—	13·8	—	14·4
ilt	12·1	10·5	—	11·7	—	9·0
	24·9	23·5	—	22·5	—	22·6
ıre	3·8	4·1	—	3·2	—	2·9
ıatter	8·2	5·6	—	6·6	—	8·7
m carb.	7·38	7·38	—	2·15	—	2·31
ıtal	94·0	93·9	—	94·95	—	94·7
nalysis:						
re	3·80	4·10	—	3·20	—	2·87
ıatter	8·20	5·6	—	6·56	—	8·70
en	·225	·202	—	·207	—	·309
residue	65·18	66·22	—	71·07	—	70·72
	·84	·80	—	·83	—	·69
	·14	·16	—	·14	—	·13
Fe_2O_3	12·87	13·49	—	13·18	—	13·26
	5·10	4·84	—	3·12	—	2·07
	·71	·55	—	·59	—	·45
l % citric						
	·028	·019	—	—	·026	·012
	·004	·006	—	—	·001	·024

Boulder Clay Soils.

	Alluvial wash with boulder clay formation			Boulder clay on greensand	
Sample	79	80	31	109	110
Stones	7·9	—	3·1	5·2	—
Mechanical analysis:		subsoil			subsoil
Fine gravel	1·8	2·9	—	2·9	3·4
Coarse sand	27·5	21·3	—	46·8	44·8
Fine sand	18·5	15·5	—	18·9	17·2
Coarse silt	10·4	—	—	9·7	—
Fine silt	7·9	—	—	5·6	—
Clay	17·9	—	—	8·7	—
Moisture	2·6	—	—	·9	—
Org. matter	6·99	—	—	5·0	—
Calcium carb.	3·15	—	2·92	·08	—
Total	96·74	—	—	98·6	—
Chemical analysis:					
Moisture	2·65	—	2·32	·90	—
Org. matter	6·99	—	5·40	5·01	—
Nitrogen	·203	—	·176	·147	—
Insol. residue	73·46	—	77·42	83·37	—
K_2O	·65	—	·70	·41	—
P_2O_5	·17	—	·14	·28	—
Al_2O_3. Fe_2O_3	11·87	—	10·19	9·50	—
CaO	2·40	—	2·42	·30	—
MgO	·44	—	·56	·30	—
Available 1 % citric acid:					
K_2O	·025	—	·022	—	—
P_2O_5	·046	—	·021	—	—

Glacial Soils.

Sample	51	52	53
Stones	5·1	8·4	6·4
Mechanical analysis:		subsoil	
Fine gravel	3·2	1·8	2·7
Coarse sand	27·7	29·8	23·3
Fine sand	22·3	21·4	17·9
Coarse silt	10·7	—	10·3
Fine silt	8·3	—	9·7
Clay	15·5	—	19·3
Moisture	2·7	—	3·7
Org. matter	6·2	—	6·05
Calcium carb.	·81	—	4·63
Total	97·4	—	97·6
Chemical analysis:			
Moisture	2·67	—	3·72
Org. matter	6·27	—	6·05
Nitrogen	·175	—	·200
Insol. residue	78·21	—	71·68
K_2O	·59	—	·76
P_2O_5	·21	—	·47
Al_2O_3, Fe_2O_3	10·23	—	11·46
CaO	1·08	—	3·47
MgO	·47	—	·57
Available 1 % citric acid:			
K_2O	·033	—	·013
P_2O_5	·024	—	·075

Brick Earth Soils.

Sample ...	23	29	30
Stones	2·5	2·4	5·3
Mechanical analysis:			subsoil
Fine gravel	1·6	1·8	2·8
Coarse sand	29·2	27·4	30·6
Fine sand	19·1	16·9	18·1
Coarse silt	15·9	11·6	11·1
Fine silt	9·4	11·2	8·7
Clay	14·9	15·4	15·5
Moisture	2·2	1·8	2·4
Org. matter	5·1	8·1	7·4
Calcium carb.	·12	1·47	—
Total	97·5	95·7	—
Chemical analysis:			
Moisture	2·25	1·82	2·42
Org. matter	5·08	8·10	7·40
Nitrogen	·165	·312	—
Insol. residue	81·39	76·52	76·48
K_2O	·50	·52	·54
P_2O_5	·12	·16	·14
Al_2O_3. Fe_2O_3	9·37	9·61	10·17
CaO	·61	1·78	2·24
MgO	·42	·45	·50
Available 1 % citric acid:			
K_2O	—	·013	—
P_2O_5	—	·033	—

Valley Gravel Soils.

	Old brown soil formation						
	Biggleswade plateau				Stanford-Broom plateau		
		90	107	108	21	87	81
Stones in soil	10·7	11·4	8·9	8·3	20·2	9·8	8·6
„ in subsoil	—	—	—	—	22·0	15·5	7·5
ıanical analysis:		subsoil		subsoil			
Fine gravel	3·3	3·7	3·4	3·1	8·5	3·7	2·6
Coarse sand	46·6	46·5	50·1	47·2	48·3	44·4	52·4
Fine sand	19·6	20·0	18·8	20·3	14·3	20·3	17·6
Coarse silt	11·1	12·0	10·0	10·1	11·2	14·2	12·1
Fine silt	5·2	5·7	5·1	5·4	6·3	3·7	3·6
Clay	5·7	6·7	6·6	8·9	5·2	7·2	5·9
Moisture	1·4	·95	·60	·7	1·0	36	1·1
Org. matter	5·1	3·25	4·3	3·0	3·7	3·6	3·5
Calcium carb.	·08	—	·03	—	·06	·19	·1
Total	98·1	98·8	98·9	98·7	98·6	97·65	98·9
nical analysis:							
Moisture	1·45	·95	·60	·68	1·00	·36	1·10
Org. matter	5·08	3·25	4·26	3·0	3·71	3·58	3·47
Nitrogen	·155	·096	·127	—	·117	·084	·097
Insol. residue	85·98	88·03	88·27	88·88	86·44	86·19	88·71
K_2O	·27	·31	·27	·31	·21	·25	·21
P_2O_5	·24	·19	·18	·15	·26	·20	·22
Al_2O_3. Fe_2O_3	6·67	6·60	6·20	6·80	8·31	7·95	6·21
CaO	·42	·36	·31	·25	·15	·49	·23
MgO	·23	·25	·26	·27	·26	·28	·19
ilable 1 % citric ːid:							
K_2O	·036	—	—	—	·015	·014	·014
P_2O_5	·090	—	—	—	·059	·050	·067

Valley Gravel Soils.

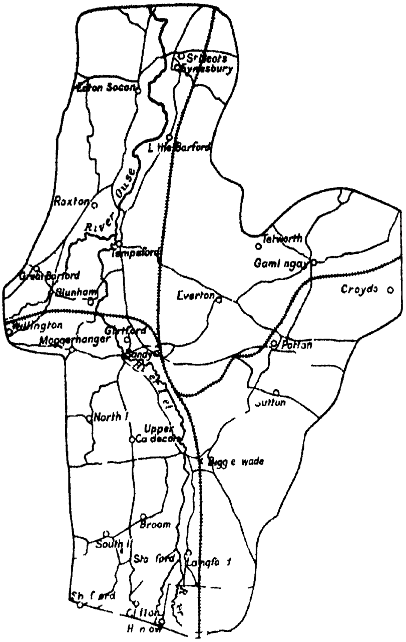
	Old brown soil formation			
	Biggleswade Common		Ouse brown gravels	
Sample ...	35	49	57	65
Stones in soil	—	4·5	10·2	23·1
„ in subsoil	9·4	7·9	9·2	29·4
Mechanical analysis:				
Fine gravel	4·5	5·3	2·4	5·8
Coarse sand	44·9	47·5	34·4	46·9
Fine sand	19·7	14·6	25·8	15·6
Coarse silt	13·3	10·8	15·0	9·2
Fine silt	5·2	6·3	6·7	6·3
Clay	6·1	3·8	8·0	6·0
Moisture	1·10	2·07	1·6	1·5
Org. matter	3·65	8·11	4·2	5·9
$CaCO_3$	·04	·07	·04	·38
Total	98·5	98·55	98·1	97·6
Chemical analysis:				
Moisture	1·10	2·07	1·59	1·46
Org. matter	3·65	8·11	4·23	5·93
Nitrogen	·127	·308	·147	·166
Insol. residue	87·74	82·76	86·81	83·48
K_2O	·26	·23	·32	·36
P_2O_5	·25	·20	·13	·33
Al_2O_3, Fe_2O_3	7·05	6·28	6·62	7·95
CaO	·23	·21	·30	·69
MgO	·24	·24	·26	·28
Available 1 % citric acid:				
K_2O	·036	016	·015	·048
P_2O_5	·059	·011	·010	·149

Valley Gravel Soils.

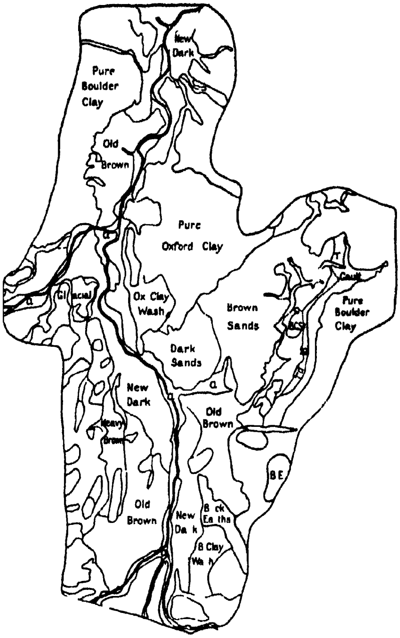
	Heavy brown soil formation	
Sample ...	105	106 subsoil
Stones	5·8	5·9
Mechanical analysis:		
Fine gravel	1·7	2·5
Coarse sand	34·1	34·5
Fine sand	21·8	20·0
Coarse silt	12·3	—
Fine silt	7·7	—
Clay	12·9	—
Moisture	·97	—
Org. matter	5·86	—
$CaCO_3$	·38	—
Total	97·7	—
Chemical analysis:		
Moisture	·97	—
Org. matter	5·86	—
Nitrogen	·165	—
Insol. residue	82·11	—
K_2O	·45	—
P_2O_5	·17	—
Al_2O_3, Fe_2O_3	9·06	—
CaO	·87	—
MgO	·42	—
Available 1 % citric acid:		
K_2O	—	—
P_2O_5	—	—

Valley Gravel Soils.

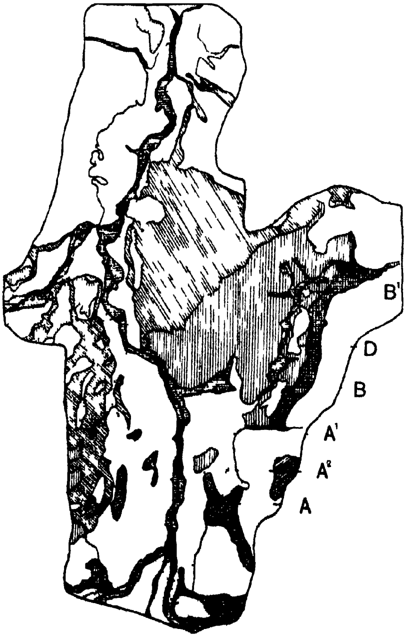
	New dark soil formation				
Sample ...	27	75	45	85	95
Stones in soil	11·1	12·2	2·9	12·5	—
„ in subsoil	13·1	13·0	—	12·2	—
Mechanical analysis:					
Fine gravel	1·9	3·9	1·2	3·7	—
Coarse sand	42·8	42·2	22·5	34·6	—
Fine sand	15·5	19·4	24·5	18·8	—
Coarse silt	10·5	10·2	18·1	12·6	—
Fine silt	7·0	5·5	8·9	6·7	—
Clay	10·3	8·9	11·3	10·7	—
Moisture	1·9	1·8	2·6	2·0	—
Org. matter	6·8	5·6	7·2	7·1	—
Calcium carb.	·43	·15	·86	·87	·16
Total	97·1	97·65	97·2	97·1	—
Chemical analysis:					
Moisture	1·95	1·80	2·62	2·02	·87
Org. matter	6·82	5·60	7·24	7·08	9·62
Nitrogen	·227	·181	·278	·238	·361
Insol. residue	81·25	83·74	78·46	79·89	80·00
K_2O	·43	·37	·38	·28	·33
P_2O_5	·37	·23	·22	·23	·27
Al_2O_3. Fe_2O_3	8·08	7·61	9·16	8·59	7·57
CaO	·94	·64	1·34	1·20	·87
MgO	·36	·33	·50	·29	·36
Available 1 % citric acid:					
K_2O	·024	·032	—	·021	·010
P_2O_5	·176	·092	—	·075	·059

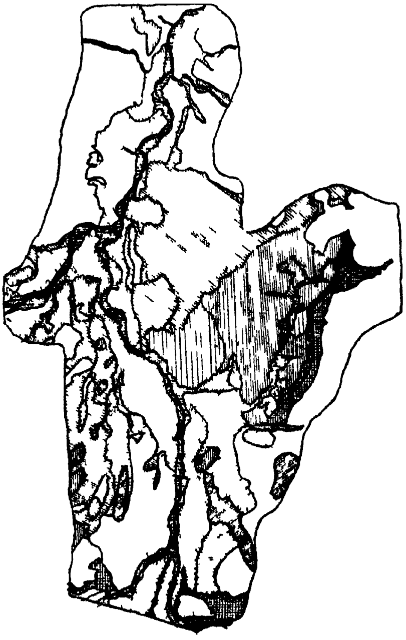


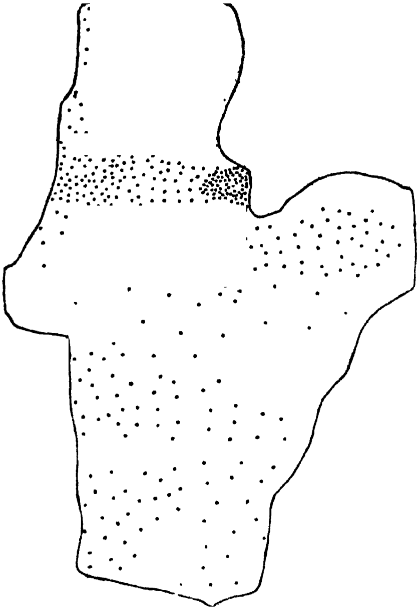
MAP 1 Topographical Map



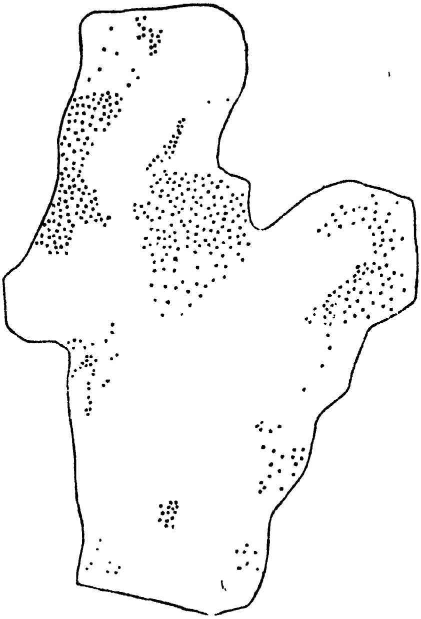
MAP 3 Outline Geological Map.
a = Alluvial *rd ld* = Redland.
B C S = Boulder clay or greensand.



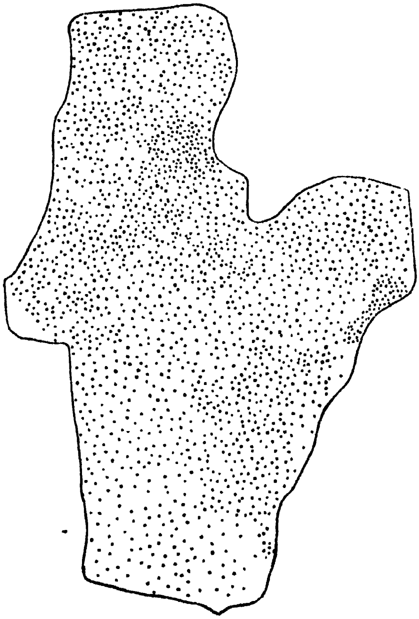




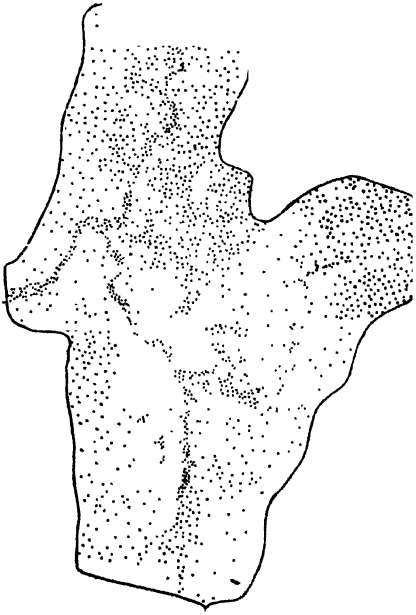
MAP 5. Horse Beans. 1 dot—1 acre
Plotted on Parish area.



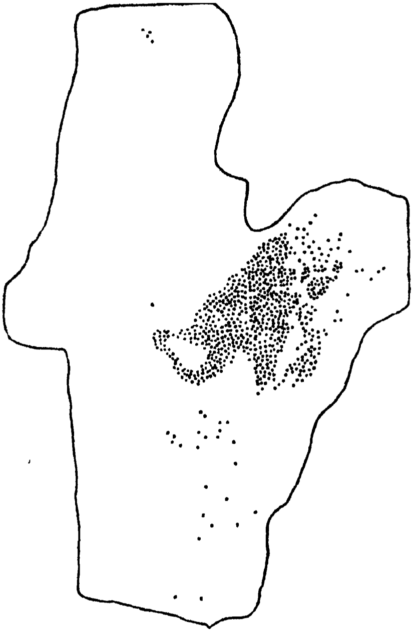
MAP 6. Horse Beans. 1 dot—1 acre.
Plotted on Soil Formations.



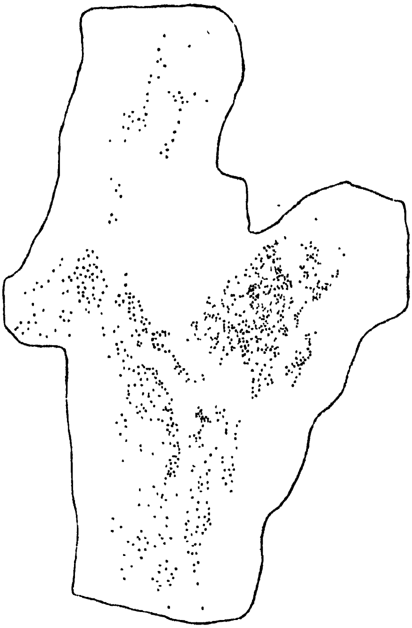
MAP 7. Pasture. 1 dot—5 acres.
Plotted on Parish area.



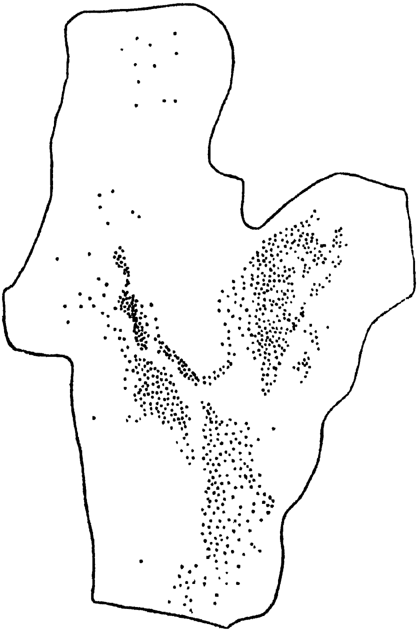
MAP 8. Pasture. 1 dot—5 acres.
Plotted on Soil Formations.



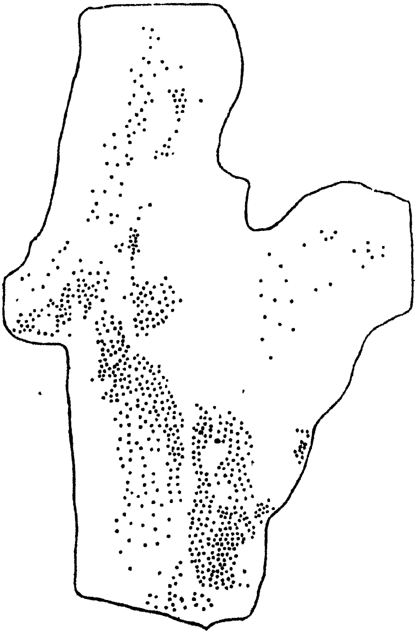
MAP 9. White Turnips. 1 dot—1 acre.



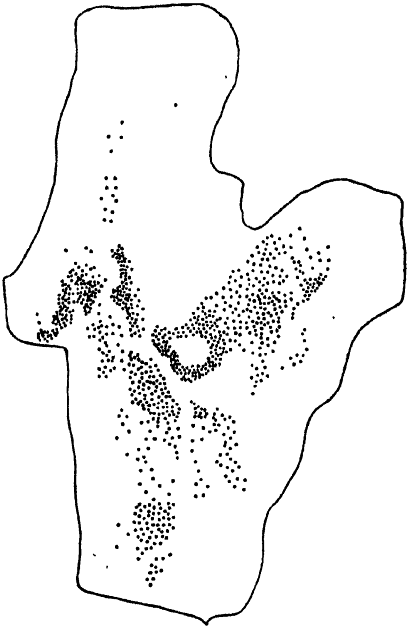
MAP 10. Early Potatoes. 1 dot—2 acres.



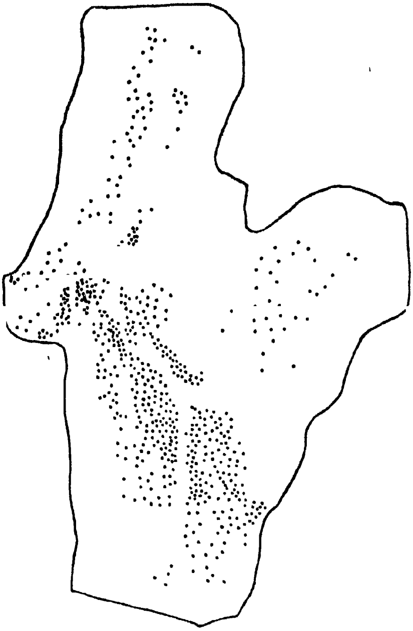
MAP 11. Carrots. 1 dot—1 acre.



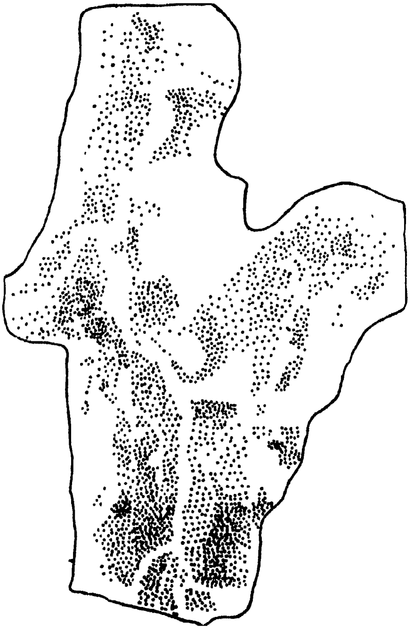
MAP 12. Onions. 1 dot—1 acre.



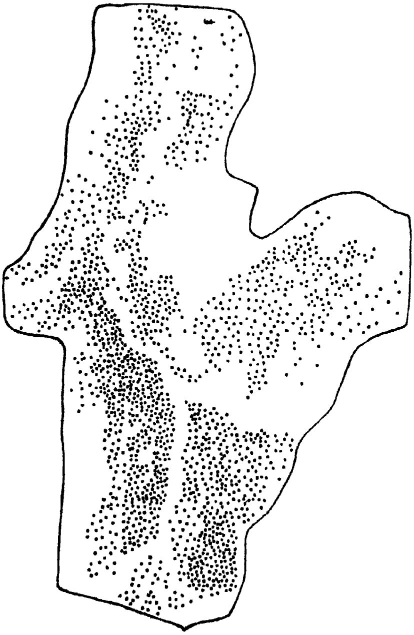
MAP 13. Runner Beans. 1 dot—½ acre



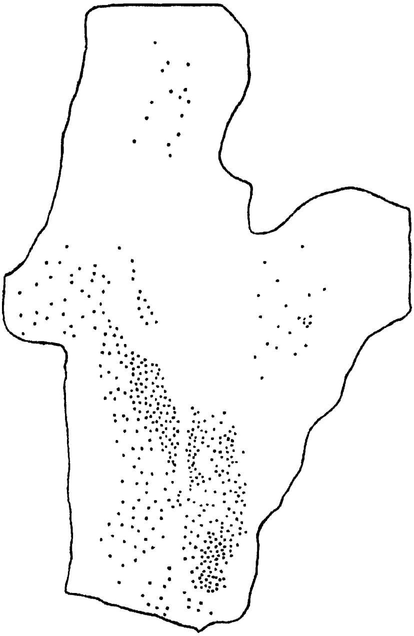
MAP 14. Parsley. 1 dot—1 acre.



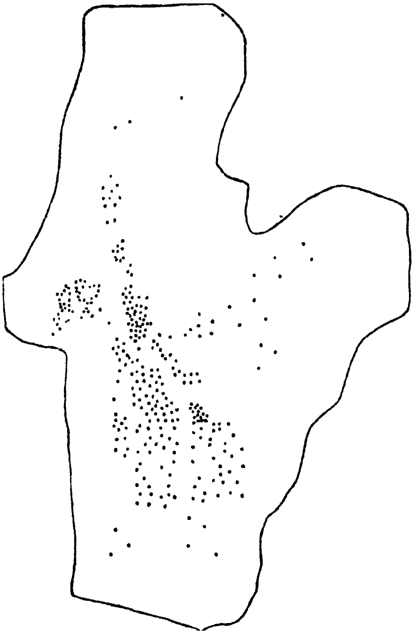
MAP 15. Late Potatoes. 1 dot—1 acre.



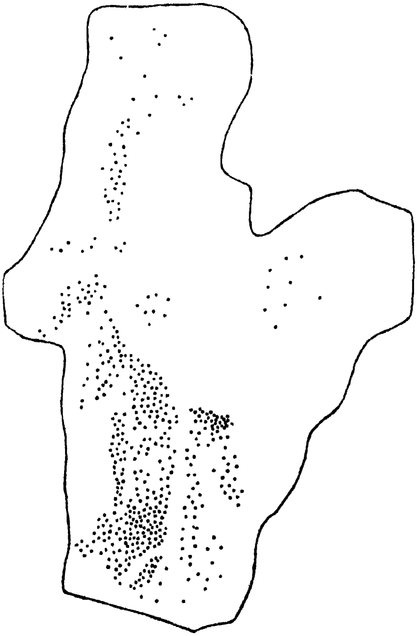
MAP 16. Brussels Sprouts. 1 dot—2



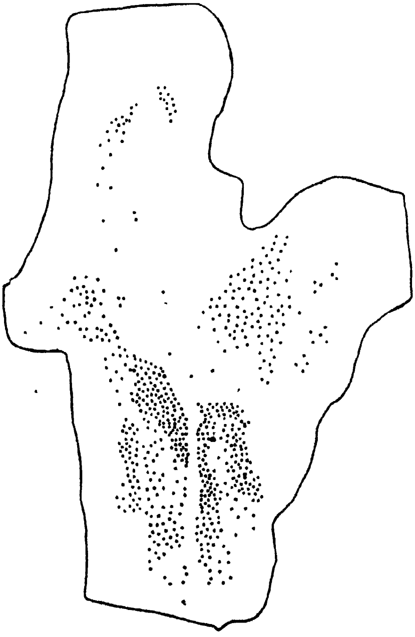
MAP 17. Parsnips. 1 dot—1 acre.



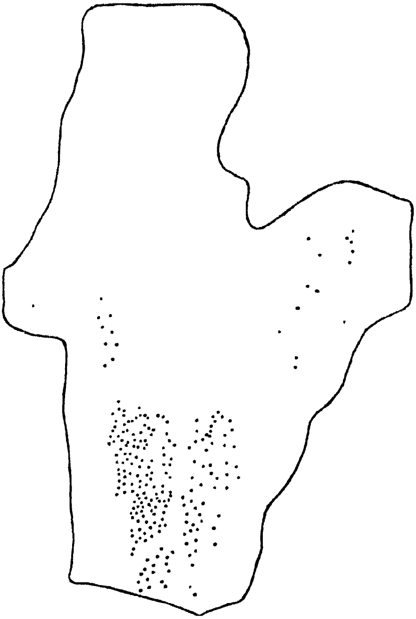
MAP 18. Marrows. 1 dot—½ acre.



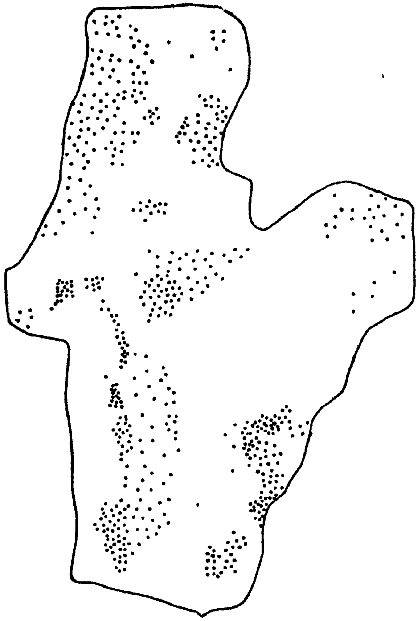
MAP 19. Green Peas. 1 dot—1 acre.



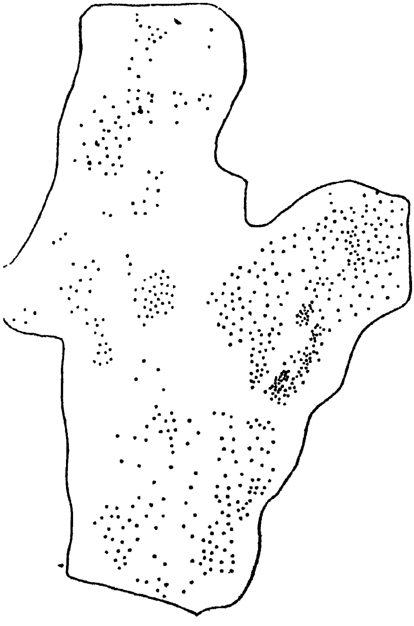
MAP 20. Spring Cabbages. 1 dot—1 acre



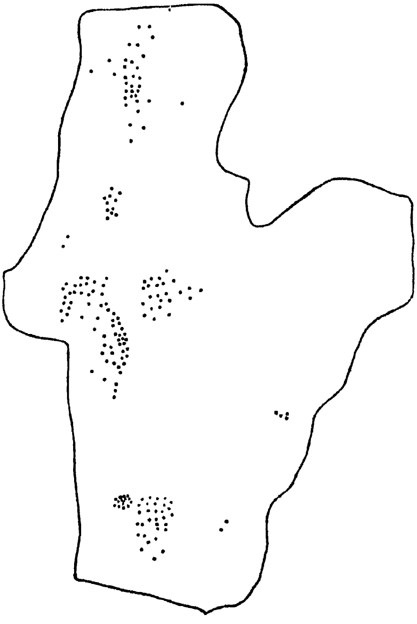
MAP 21. Cauliflowers. 2 dots—1 acre.



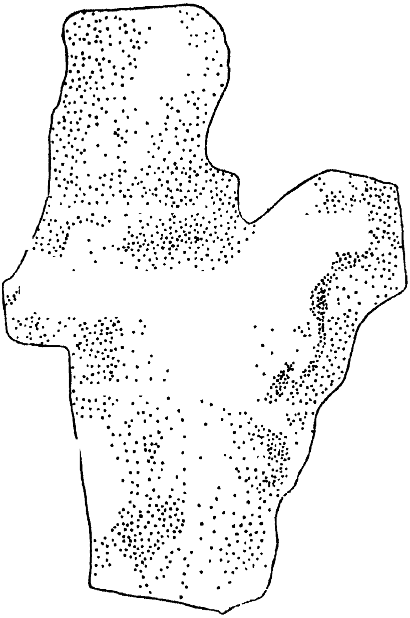
MAP 22. Mangel Seed. 1 dot—1 acre.



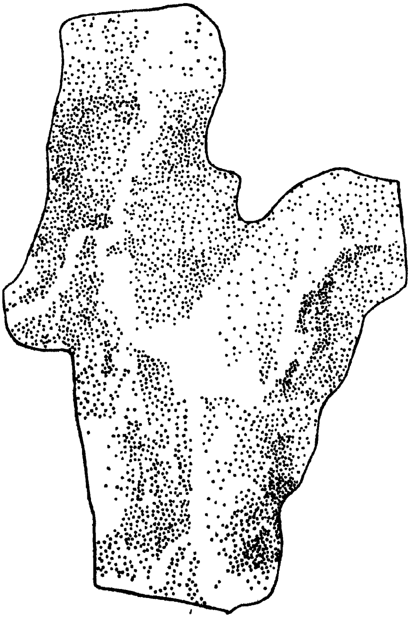
MAP 23. Roots. 1 dot—1 acre.



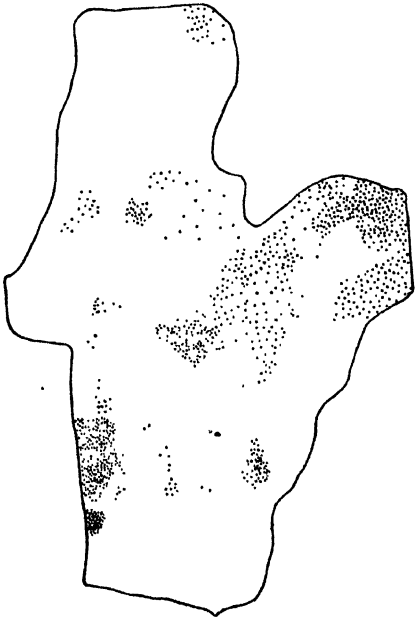
MAP 24. Small Seeds—Carrot, Turnip, Onion, Parsley, Brussels Sprouts, Kohlrabi. 1 dot —1 acre.



MAP 25. Legumes. 1 dot—1 acre.



MAP 26. Cereals. 1 dot—3 acres



MAP 27. Parks and Plantations 1 dot—3 acres.

(*Received October 7th,* 1915.)

THE RELATION OF CERTAIN PHYSICAL CHARACTERISTICS OF THE WHEAT KERNEL TO MILLING QUALITY.

By C. H. BAILEY.

(Division of Agricultural Chemistry, Minnesota Agricultural Experiment Station, St Paul, Minn.)

The production of flour from wheat by the roller milling process is a series of mechanical operations which have as their final object the separation of the fibrous pericarp and the germ from the endosperm, and the reduction of the latter to a fine powder. The more exact the separation of these structures, the more desirable the process. In actual practice a quantitative separation is never obtained, since a plump wheat kernel may be from 82 to 85 per cent. endosperm[1], and yet only from 72 to 75 per cent. of the kernel is ordinarily recovered as flour. The other 10 or 12 per cent. adheres closely to the pericarp and germ fragments and is lost in the by-products or feeds. Since flour is the most valuable product of milling, it follows that those wheats which will yield the highest percentages of flour possess the greatest intrinsic value. The term "milling quality" is accordingly used by the writer in this paper to express the potential yield of edible flour when milled by the usual roller process.

When the same general system of milling is employed the relative yields of flour will depend, other thing being equal, upon the percentage of endosperm in the kernels. The quantitative determination of the proportion of the several kernel structures is difficult, since it involves the dissection of a sufficient number of kernels to furnish both a fair average sample and a quantity of material which can be satisfactorily weighed. There is a relation between the volume or displacement of the kernel and the proportion of endosperm, however, of which advantage may be taken. As shown by Brenchley[2], the pericarp and germ are

[1] Hunt. *Cereals in America*, p. 21, New York, 1908.

[2] Brenchley. *Ann. Bot.* **23**, 117–139, 1909.

formed largely during the early stages of the development of the kernel, and the endosperm structure is rapidly laid down during a comparatively short time preceding maturity. Any condition which interferes with the deposition of material in the endosperm during the later stages of kernel development reduces the quantity of potential flour material without reducing the amount of fibrous seedcoat in like proportions. A plump well-filled kernel accordingly yields more flour than does a shrivelled one. The comparison on the basis of kernel volume must be restricted to the same type or variety, since hereditary influences affecting the shape of the kernel, particularly its length, would affect the volume and weight without similarly affecting the ratio of endosperm to total weight.

To ascertain the relation between the kernel volume and the actual percentage of endosperm, samples were taken from a field of blue stem wheat at six stages of growth, beginning about ten days after flowering and at intervals of three days thereafter until the grain was nearly ripe enough to harvest. The kernels were at once removed from the heads, and the endosperm material dissected out of 100 kernels. The non-endosperm structures were dried to constant weight and 300 entire kernels were similarly dried. The difference in the weight of the kernels and of the non-endosperm material was regarded as the weight of the endosperm, since the latter was of such a sticky character that a loss was experienced in collecting it. The volume of the dried kernels was then determined by displacement in toluol. Table I shows the percentage of endosperm to increase fairly regularly from 62·6 per cent. when the kernels occupied a volume of 9·897 c.c. per 1000, to 81·7 per cent. when the kernel volume was 21·372 c.c. per 1000.

TABLE I. *Relation between the kernel volume and the weight and percentage of endosperm.*

Volume per 1000 kernels c.c.	Endosperm per 1000 kernels grams	Endosperm Per ct.
9·897	0·7950	62·6
12·317	1·1443	66·6
16·114	1·5682	69·8
17·871	1·7826	71·2
19·417	2·1275	76·9
21·372	2·4576	81·7

The volume of the kernel may vary widely in the same type or variety of wheat when grown under different conditions. Thus hard spring wheat samples grown in Minnesota have been examined which displaced from 7·2 to 25·2 cubic centimetres per 1000 kernels. Greater extremes than these would doubtless be found in working with a larger number of samples. These variations are due to environmental influences, particularly those which affect the rate of photosynthesis just preceding the ripening of the plant. Soil moisture, humidity, sunshine, temperature, and winds are most potent in this regard. Severe rust and scab infections result in the production of shrivelled, light-weight kernels.

Similarly there are wide variations in the percentage of flour which can be produced from wheats of varying plumpness. In estimating the relative percentage of flour which can be milled from different samples of wheat, the kernel volume and the density are believed to be better indices than the weight per bushel. The latter is influenced not only by the actual volume of the kernels but by their relative length: width ratio, and other factors affecting the manner in which they pack together. While the relation of the two factors mentioned to milling yield is not exact in all cases, certain experiments which we have made show them to be of considerable value in estimating the quality of wheat from this standpoint.

Table II. *Physical characteristics of composite samples of northern spring wheat grades and the yield of flour obtained from them in milling.*

Lab. No.	Grade	Specific gravity	Volume per 1000 kernels c.c.	Flour yield Per ct.
C 835	No. 1 northern spring ...	1·4218	16·10	70·0
C 836	„ 2 „ „ ...	1·4112	14·31	69·1
C 837	„ 3 „ „ ...	1·4212	12·97	66·9
C 838	„ 4 „ „ ...	1·4238	10·50	65·2

Table II shows the results of one series of tests made to determine the relation of kernel volume to the percentage of total flour obtained in milling. A fairly regular decrease in flour yield was observed as the kernels decreased in size. The samples employed in this case were composite No. 1, No. 2, No. 3, and No. 4 northern spring wheat, representing the average of these grades in the Minnesota markets. Similar results were obtained in milling other wheat samples of varying plumpness. The milling tests were made in the experimental roller mill at this station. The method employed is described in Minnesota Agricultural Station Bulletins, Nos. 131 and 143. While the flour yields obtained with this small equipment are not identical with those obtained by large merchant mills working on the same materials, they are comparative with each other if the experimental mill is properly handled.

The other two physical characteristics which bear the most definite relation to milling value are the density of the kernel, more particularly that of the endosperm, and the moisture content. The moisture content is considered as a physical rather than a chemical characteristic since it can be altered by purely physical means. An increase in moisture content above the normal results in increased losses through evaporation, as has been shown by the writer[1]. In addition, damp wheat presents certain mechanical difficulties in milling, owing to its soft character, which render more difficult the separation of the bran and endosperm. For these reasons, and because of the increased liability of spoilage through fermentation and heating, damp wheat is of less value to the miller than dry wheat.

The density of the endosperm of the wheat kernel is known to vary widely. These variations are commonly distinguished as "mealiness" in the case of the less dense, and "flintiness," or a "vitreous," "horny" or "corneous" condition in the case of the more dense. The light-coloured condition of the endosperm frequently met with in the hard red wheats is usually referred to as "yellow-berry"; when the entire kernel is not affected it is sometimes called "piebald." These variations in endosperm density have been the subject of numerous investigations. Nowacki[2] states that the difference in appearance of mealy and horny wheat kernels is due to the presence of a larger volume of air-spaces in the former. Hackel[3] (p. 26) says, "If the albuminoids so fill up the intervals between the starch grains that the latter seem to be imbedded in cement, the albumin appears translucent and the fruit is called corneous; but if the union is less intimate, there remain numerous small air-cavities and the albumin is opaque and the fruit is mealy. Both conditions may occur in the same species or variety (wheat) and they seem to be occasioned by differences in climate and soil. Corneous fruits are usually richer in albuminoids than mealy ones of the same species." Pagnoul[4] and Wollny[5] found that the specific gravity of

[1] Bailey. *Canadian Miller and Cerealist*, vol. VI. pp. 74–75, 1914

[2] Nowacki. *Untersuchungen über das Reifen des Getreides*, Halle, 1870.

[3] Hackel. *The True Grasses*. Translation by Lamson-Scribner and Southworth, New York, 1890.

[4] Pagnoul. *Ann. Agron.* vol. XIV. pp. 262–272, 1888.

[5] Wollny. *Forsch. a. d. Gebiete Agrikulturphysik*, vol. IX. pp. 207–216, 1886.

the wheat kernel increased with the nitrogen or protein content. Marek[1] states that in the samples examined by him a decrease in nitrogen content was accompanied by an increase in specific gravity. Kornicke and Werner[2] state that the constituents of the wheat kernel have the following specific gravity: starch, 1·53; sugar, 1·60; cellulose, 1·53; fats, 0·91–0·96; gluten, 1·297; ash, 2·50; air, 0·001293. They further state that the volume weight bears no relation to specific gravity or to protein content. Lloyd[3] determined the weight per 100 kernels,volume weight, and densities of wheats from different parts of the world and found Russian wheat to possess the lowest average weight per kernel, and Australian wheat the highest. Wheat grown in the United States and Canada had the highest average density or specific gravity, and that grown in England the lowest. Pammel and Stewart[4] found the specific gravity of wheat examined by them to range between 1·407 and 1·503. Lyon[5] states that wheat kernels having a high percentage of proteid material have a lower specific gravity. His data (p. 57) indicate that large kernels have a higher specific gravity than small kernels of the same variety. Cobb[6] observed that there are fewer large starch granules in wheats containing a low percentage of nitrogen. Lyon and Keyser[7] confirmed Cobb's observations, and also found that large and numerous vacuoles are associated in yellow-berry kernels. The difference in structure between horny and yellow-berry kernels was also accompanied by a difference in nitrogen, the yellow-berry kernels containing less nitrogen than the horny. Roberts[8] states that the presence of air vacuoles doubtless accounts for the lower specific gravity of yellow-berry kernels. He later[9] presented the results of a number of physical measurements of wheat kernels, including specific gravity. The samples examined by him ranged in specific gravity from 1·218 to 1·386. Willard and Swanson[10] determined the specific gravity and other factors of quality for Kansas, Minnesota, Tennessee, and Washington wheats. They state (p. 81) that there is a tendency for large kernels to have the higher specific gravity, and further, that small, compact kernels have a higher specific gravity than the large (and presumably less compact) ones.

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[1] Marek. *Landw. Zeit. f. Westfalen u Lippe*, p. 362, 1875.
[2] Kornicke and Werner. *Handbuch d. Getreidebaues*, Berlin, 1884.
[3] Lloyd. *Amer. Journ. Pharm* vol. LXVI. pp. 413–419, 1894.
[4] Pammel and Stewart. *Iowa Exp. Sta. Bul.* No. 25, pp. 26–31, 1894.
[5] Lyon. *U.S. Bur. Plant Ind. Bul.* No. 78, 1905.
[6] Cobb. *Agr. Gaz. New South Wales*, vol. XV. p. 512, 1904.
[7] Lyon and Keyser. *Nebraska Exp. Sta. Bul.* No. 89, 1905.
[8] Roberts. *Kansas Exp. Sta. Bul.* No. 156, 1908.
[9] Roberts. *Kansas Exp. Sta. Bul.* No. 170, 1910.
[10] Willard and Swanson. *Kansas Exp. Sta. Bul.* No. 177, 1911.

The methods previously employed for the determination of specific gravity did not appear in most instances to be wholly satisfactory. A new method was accordingly developed by the writer in collaboration with L. M. Thomas[1]. About 10 grams of wheat kernels, freed from dirt, weed-seeds, other grains, and broken kernels, are weighed on an analytical balance, and the exact weight recorded. The wheat is then placed in a 50 c.c. pycnometer, which is provided with a ground-in thermometer, side-tube, and over-flow cap. The grain is just covered with cool toluol, the side-tube plugged, and the neck of the pycnometer connected with pressure tubing to an aspirator. The air is then exhausted to free the mass of grain from air mechanically held in the brush and crease of the kernels. Unless this is done the air so held will materially reduce the apparent specific gravity. Moreover the quantity of air present and removed by aspiration varies, as it depends upon the shape and size of the kernels. In nine trials it ranged from 0·101 c.c. to 0·335 c.c. After the bubbles cease to rise through the toluol, air is slowly admitted, and the pycnometer is disconnected from the aspirator and completely filled with toluol at a temperature of about 18° C. The temperature is allowed to rise slowly to 20°, as shown by the thermometer in the pycnometer, the last drop on the side-tube is wiped off, and the over-flow cap set firmly in place. Pycnometer and contents are then weighed on the analytical balance. The exact capacity of the pycnometer and the specific gravity of the toluol must of course be known. The latter averages about 0·8665. The specific gravity of the wheat is calculated according to the following formula:

$$\text{specific gravity of wheat} = \frac{\text{specific gravity of toluol} \times \text{weight of wheat}}{\text{weight of displaced toluol}}.$$

From the weight of toluol displaced by the wheat its volume can be calculated and this figure divided by the number of kernels in the pycnometer gives the average volume per kernel. From the weight of wheat in the pycnometer the weight per 1000 kernels can also be calculated when the number of kernels is known.

These investigations have shown the kernel density to be dependent first upon the proportion of pericarp and germ to endosperm, and second upon the density of the endosperm. As a general rule the small kernels, which have the larger proportion of bran and germ, also have the lower specific gravity. This is shown by Table III, which gives the specific

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[1] Bailey and Thomas. *U.S. Bur. Plant Ind. Circular* No. 99, 1912

TABLE III. *Relation of specific gravity to kernel volume.*

Lab. No.	Specific gravity	Volume per 1000 kernels, c.c.	Nitrogen Per ct.
Less than 12 c.c. per 1000 kernels.			
C 784	1·3895	7·21	2·30
C 789	1·3934	10·86	2·12
C 783	1·4083	10·97	2·62
C 795	1·3862	11·72	2·18
C 796	1·3607	11·78	2·16
Average	1·3876	10·51	2·28
From 12 to 14 c.c. per 1000 kernels.			
C 782	1·4022	12·74	2·28
C 789	1·4016	13·75	2·54
C 791	1·4033	12·35	2·86
C 793	1·4099	12·98	2·39
Average	1·4042	12·95	2·52
Over 14 c.c. per 1000 kernels.			
C 786	1·4101	14·48	2·07
C 790	1·4104	14·71	2·35
C 794	1·4233	15·83	2·44
C 792	1·4212	18·54	2·47
Average	1·4162	15·89	2·33

gravity of a number of hard red spring wheat samples arranged according to their size or kernel volume. Deviations from the general rule exhibited by certain of these samples may be explained on the basis of their nitrogen content, as will be shown later. Thus C 783 in the first group has a specific gravity of 1·4083, which is somewhat above the average of similar samples so far as kernel volume is concerned. It contains 2·62 per cent. of nitrogen, however, which is considerably more than is found in the others in the group. Similarly the average specific gravity of the entire second group, in which the kernel volumes range between 12 and 14 c.c. per 1000, is probably somewhat high because of the higher average nitrogen content. The data indicate that the bran and germ tissues have a lower specific gravity than do the endosperm structures, which fact must be considered in evaluating wheats on the basis of specific gravity.

Practical flour millers have observed that in general the milling of the soft types of wheat by the roller process presents greater difficulties than does the milling of hard kinds of the same degree of plumpness.

The flour middlings, or endosperm particles, are more difficult to separate from the bran. Their subsequent reduction to flour between the smooth rolls is not accomplished as easily as when they were produced from harder kernels, owing to their tendency to flatten out or "flake," and lose their granular character before the reduction is complete. There is in consequence an increased loss of endosperm or floury material in the feeds or by-products. Increasing the length of the milling system aids somewhat in effecting a more complete separation but involves greater expense of operation.

TABLE IV. *Physical characteristics, flour yield, and nitrogen content of vitreous and mealy samples of hard red spring and hard red winter wheats.*

Lab. No.	Total nitrogen Per ct.	Volume per 1000 kernels c.c.	Specific gravity	Flour yield Per ct.
		Vitreous spring wheats.		
C 195	2·79	20·58	1·4185	71·2
C 317	2·29	22·94	1·4233	69·1
C 294	2·46	19·56	1·4180	69·6
C 321	2·30	20·94	1·4251	73·0
C 298	2·56	19·70	1·4184	72·3
Average	2·48	20·74	1·4207	71·0
		Mealy spring wheats.		
C 308	1·82	18·60	1·4106	70·6
C 338	1·92	24·17	1·3988	68·8
C 353	2·02	20·56	1·4015	69·7
C 372	1·89	22·98	1·4031	67·7
C 396	1·99	20·65	1·4174	69·6
Average	1·93	21·39	1·4063	69·3
		Vitreous winter wheats.		
C 263	2·31	18·63	1·4277	67·8
C 631	2·10	20·68	1·4262	72·7
C 567	2·25	21·45	1·4225	71·7
C 616	2·25	23·92	1·4129	71·7
C 673	2·42	23·97	1·4244	71·2
Average	2·27	21·73	1·4227	71·0
		Mealy winter wheats.		
C 239	1·62	23·10	1·4051	70·8
C 247	1·52	19·98	1·4109	69·9
C 249	1·66	20·82	1·4022	66·8
C 252	1·49	20·54	1·4101	64·7
C 260	1·63	21·77	1·4000	68·2
C 265	1·64	22·51	1·3990	66·9
C 289	1·64	21·59	1·3964	66·2
Average	1·60	21·47	1·4034	67·6

No exact information was available on the relation of kernel density to milling yield when the same general system of milling was followed in all cases. There were on file in this laboratory several hundred wheat samples which had been analysed, and milled in a 5-break and 8 to 11 reduction system. From these were selected at random twenty-two samples of both hard red spring and hard red winter (Turkey Red)

TABLE V. *Physical characteristics and nitrogen content of soft red winter wheats grown in the eastern United States.*

Lab. No.	Source	Specific gravity	Volume per 1000 kernels c.c.	Nitrogen Per ct.
	Less than 1·80 per cent. of nitrogen.			
C 727	St Mary's County, Maryland	1·3393	26·89	1·50
C 723	Talbot County, Maryland	1·3303	25·75	1·53
C 721	Northumberland County, Maryland	1·3401	26·02	1·58
C 725	Westmoreland County, Maryland	1·3432	27·75	1·60
C 722	St Mary's County, Maryland	1·3444	27·10	1·60
C 724	Queen Anne County, Maryland	1·3633	30·22	1·62
C 720	Talbot County, Maryland	1·3419	25·99	1·64
C 716	Dorchester and Caroline Co., Md.	1·3804	28·06	1·64
C 717	Queen Anne County, Maryland	1·3568	26·35	1·66
C 729	Washington, Indiana	1·3728	35·07	1·68
C 719	Queen Anne County, Maryland	1·3592	24·12	1·74
C 718	Queen Anne County, Maryland	1·3606	31·28	1·75
C 728	Washington, Indiana	1·3898	22·92	1·79
	Average	1·3556	27·50	1·64
	More than 1·80 per cent. of nitrogen.			
C 741	Lyndon, Ohio	1·3818	24·86	1·82
C 739	Washington, Indiana	1·3981	25·93	1·84
C 731	New Vienna, Indiana	1·3856	22·72	1·90
C 743	Arcanum, Ohio	1·4083	27·29	1·90
C 737	Thrifton, Ohio	1·4070	25·18	1·94
C 734	Xenia, Ohio	1·4019	22·09	1·98
C 732	Washington C. H., Ohio	1·4155	22·23	2·00
C 738	Elmora, Indiana	1·4025	23·87	2·00
C 730	Madison Mills, Illinois	1·3866	23·56	2·01
C 740	Chillicothe, Ohio	1·3900	22·97	2·04
C 733	Derby, Ohio	1·4106	24·42	2·11
C 736	Markelville, Indiana	1·4084	24·18	2·14
C 735	McCords, Indiana	1·4014	24·82	2·18
	Average	1·3998	24·16	2·00
	Average of both groups	1·3777	25·83	1·82

wheat of approximately the same degree of plumpness, but varying in colour and hardness. The soft, mealy samples represented what is commonly termed "yellow-berry." The kernel volume and specific gravity of these samples were then determined and the results of these tests, the flour yields, and the percentages of total nitrogen are shown in Table IV. The data are grouped according to type of wheat, i.e., winter or spring, and into two sub-groups in each case according to relative hardness. While there is some overlapping in the case of the flour yields from the vitreous and mealy samples, the general tendency was decidedly in the direction of larger flour yields from the vitreous grain. The relation between density or specific gravity and percentage of nitrogen is also marked, the samples having a lower specific gravity almost invariably having a low nitrogen content as well, when the comparison is restricted to kernels of about the same volume or plumpness.

For comparison with the hard wheats of the northern Great Plains area, a number of samples of soft red winter wheats grown in the eastern half of the United States were secured through representatives of the Office of Grain Standardization of the United States Department of Agriculture. The results of the tests of these samples are given in Table V. They are arranged in two groups, those having a nitrogen content of less than 1·80 per cent. being included in the first group, and those of 1·80 per cent. or over in the second group. These soft red wheats have a lower average specific gravity and nitrogen content than the hard wheats, although the average kernel volume is greater. The same relation between nitrogen content and specific gravity prevails here as did in the case of the hard wheats studied, viz. the higher the nitrogen content, the greater the specific gravity, as a general rule.

SUMMARY.

Kernel volume, because of its relation to the ratio of endosperm to non-endosperm structures, varies directly with the potential flour yield when comparisons are restricted to the same type or variety of wheat.

Accurate determination of kernel density must include the complete removal of all mechanically held air.

Large kernels, other things being equal, have a higher specific gravity than small kernels of the same variety, indicating the endosperm to have a higher specific gravity than the bran and germ.

Relative density of the endosperm is generally conceded to be dependent upon the proportion and size of the air vacuoles. Soft, light-coloured, yellow-berry kernels have a lower specific gravity than hard, dark-coloured kernels of the same variety. The more dense the endosperm, other things being equal, the greater the ease of, and the more complete, the separation of endosperm from bran and germ in milling.

Wheat kernels of a high specific gravity have a higher nitrogen content as a usual thing than less dense kernels of the same relative size or volume.

Hard red wheats grown in the northern Great Plains area, while varying widely, have a higher average specific gravity than do the soft red winter wheats grown in the eastern half of the United States.

(*Received September 18th,* 1915.)

SOME EXPERIMENTS ON THE HOUSE-FLY IN RELATION TO THE FARM MANURE HEAP.

By H. ELTRINGHAM, M.A., D.Sc.

(Rothamsted Experimental Station, Harpenden.)

In the early part of the present year the suggestion was made to me by my friend Mr J. C. F. Fryer that I should carry out a series of experiments designed to test the fly-breeding capacity of the open farm manure heap as distinguished from heaps in close proximity to dwellings.

Of late years the danger of the fly pest has been made clear to the most unscientific of the public and in many directions determined efforts have been made to reduce the number of flies infesting houses, thus minimising not only the physical nuisance, but also a far more important factor, the danger entailed by the flies' power of carrying disease.

It is unnecessary here to set forth the proofs which have been obtained of the capacity of flies to convey bacteria, or the methods by which they distribute the organisms they carry and contain. Those who desire to learn the details of a somewhat unpleasant study should read Dr L. O. Howard's work, *The House-Fly—Disease Carrier.* There they will find the whole subject set forth in a manner which leaves the reader wondering which to admire more, the completeness of the work or the literary skill which can make so unsavoury a subject both readable and entertaining. Neither is this the place to summarise the excellent work carried out by many investigators amongst whom the names of Dr Graham Smith, Dr L. O. Howard, Dr C. Gordon Hewitt, Professor Maxwell Lefroy, Professor R. Newstead, and others, will immediately occur to those who are interested in these matters. Dr C. Gordon Hewitt's volume on the House-Fly is the standard work on the anatomy and life-history of the insect.

I have before me a list of over 100 authors of volumes and papers dealing with the subject. With so formidable an array of literature available it may be asked what further point remains to be elucidated. All are agreed that house-flies breed in almost any refuse and particularly in manure. So certain and universally accepted is this fact that it has induced an almost equally general converse belief that all manure breeds house-flies.

The object of my research has been to ascertain to what extent this latter hypothesis could be supported by actual experiment. I am indebted to Dr E. J. Russell, the Director of the Rothamsted Experimental Station, for providing every facility for carrying out the work under the most favourable conditions, and to the Board of Agriculture for a grant towards the personal and material expenses incurred.

I should like to take this opportunity of thanking all the members of the Staff of the Rothamsted Laboratory for their many kindnesses, and especially would I express my gratitude to my friend Mr E. H. Richards, who not only assisted me in much of the practical work of erecting the apparatus but also attended to the experiments during a short period when I was incapacitated owing to a slight accident.

I am also greatly indebted to my friends Mr J. E. Collin and Mr A. H. Hamm for assistance in naming some of the flies observed in the course of the experiments.

The farm manure heap may be purely stable manure or it may be mixed refuse containing the excreta of other animals in addition to horses. Stable manure is usually stored light, i.e. it is not trodden down and compacted, whilst mixed manure may be stored either light or compacted. Such heaps may be out in the open at a considerable distance from dwellings, or they may be quite near to a house or houses. Neither of these conditions resembles the state of affairs which obtains when a stable manure heap occurs in a confined space in a town, and is closely adjacent to many houses and shops, with kitchens, bakeries, etc., in the immediate neighbourhood.

To reproduce the two former conditions suggested above, together with the different nature and treatment of the manure, six experimental heaps were established. Three of these were placed on land adjoining the laboratory, and their relation to their surroundings may be gathered from the accompanying sketch (Fig. 1). The experimental heaps are there marked 1, 2, and 3. They were established on ground forming part of an area which is used by the laboratory for out-door experiments. The nearest dwellings are the cottages shown at a distance of some

220 feet, whilst a hen-run, two garbage heaps, containing principally vegetable refuse, a water closet and an earth closet, are shown at the distances respectively indicated. The main street of the village of Harpenden is some quarter of a mile distant, so that the experiments conducted at the laboratory may be regarded as having been carried on under semi-rural conditions.

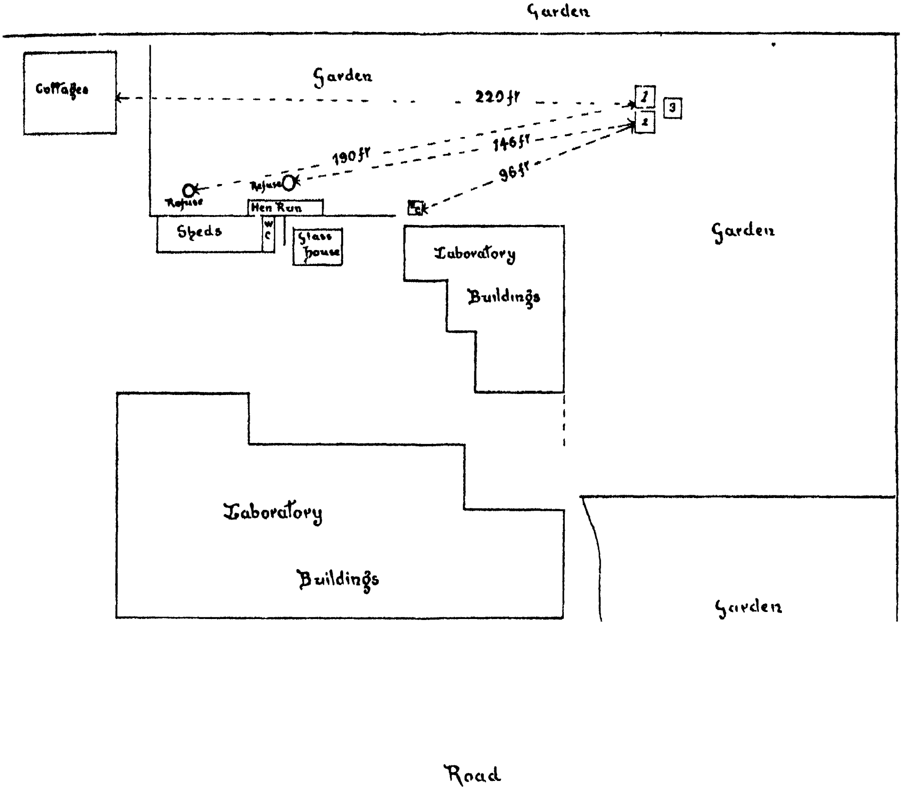

Fig. 1.

It has been found that under certain circumstances the larvae of the flies tend to leave the heaps and pupate in the ground round about so that many flies might escape if the heap alone were enclosed. The traps were therefore designed so as to provide a suitable pupating ground, to limit the lateral wandering of the larvae, and to trap any flies which might emerge from the ground surrounding the heaps.

Reference to Fig. 2 will make the design clear. The drawing represents a section of one of the experimental heaps. A rectangular trench *G*, about a foot deep and 18 inches wide, was first made and the outer sides lined with a suitable structure consisting of boards attached at the corners to stout posts. The trench was then filled up to the original ground level with a mixture of loose earth and old manure straw forming a light mass suitable for the pupation of the larvae. The extreme hardness of the ground at Harpenden together with the outer lining of wood made it improbable that the larvae would wander beyond this trench. Resting on the outer edge of the original block of ground *F* was a wood frame about 12 inches deep and 5 feet square, *A*.

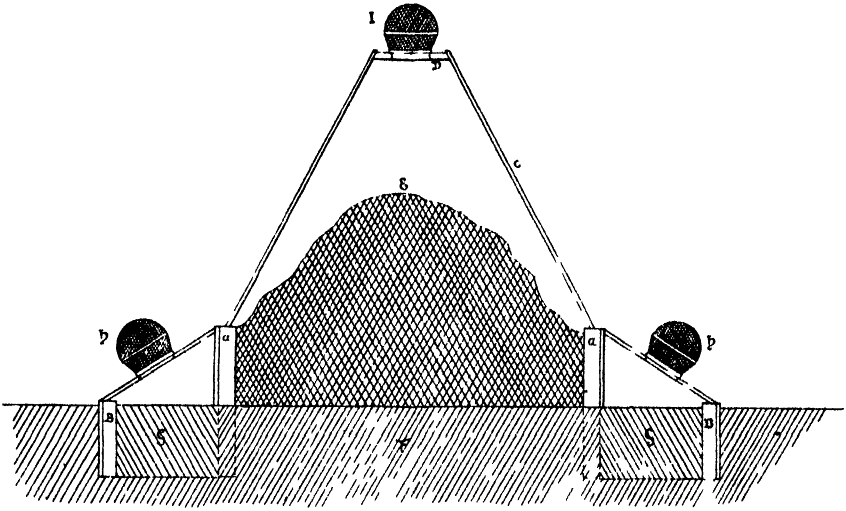

Fig. 2.

This served as a container for the manure *E*. The trench *G* was covered in with unbleached cheese cloth attached to the inner and outer frames, and on strips of wood having central holes, ordinary wire balloon traps were fitted with a sliding arrangement enabling them easily to be removed and replaced. At the four corners of the inner frame stout canes were set up and joined to a small board *D*, which also carried a fly trap. A tent-like cover of cheese cloth was then made to fit over this framework and was fastened down with tape and tacks all round the inner wood frame. In this way it was possible to note the numbers and kinds of flies which hatched from the heap proper and from the trench respectively. No bait was used, it being found that the phototropic tendency of the flies was sufficient to cause them to enter the traps, and it is certain that very few failed to do so. The general appearance of the completed traps is shown in the photograph herewith, which represents those on the laboratory ground. It may be remarked that unbleached cheese cloth is superior to the bleached quality, in that it is cheaper, stronger, and transmits less light.

Fig. 3.

Experiment I. On June 22nd a heap was started at the laboratory, one barrow load of horse manure from neighbouring army stables being placed upon it daily. By the 25th the temperature of the centre of this heap had risen to 75° (all temperatures are given on the Centigrade scale) and on the 28th it had fallen to 65°. The temperature fell slowly to 54° on July 6th, when the heap was completely covered in, the quantity of manure being estimated at about 14 cwt. On July 8th the temperature of the heap was 47°, and on the 9th two flies were found in the top trap, one being *M. domestica.* On July 11th one more of the same species hatched out. On July 21st the temperature of the heap was 20°, and on the 26th 23°. On July 29th the heap was opened out and cleared away. The total flies of all kinds which hatched from this heap numbered 16 and were of the following species:

Eristalis tenax L.	1
Musca domestica L. ...	3
Stomoxys calcitrans L. ...	5
Fannia canicularis L. ...	1
Chortophila cilicrura Rdi. ...	4
Hydrotaea armipes F. ...	2

The result does not support the belief that any of the flies taken were breeding to an appreciable extent in the material used. The manure had ample opportunity of becoming infected either at the army stable or at the laboratory. During this period *M. domestica* was not by any means commonly observed in the neighbourhood.

Experiment II. As a control and in order to discover whether there was anything in the experimental method which might inhibit the growth and development of the larvae, a quantity of horse manure was obtained from a stable closely surrounded by houses and adjacent to a bakery. This material was treated precisely as in Experiment I, except that it was all put on the heap at once and immediately covered in. This was done on July 30th. By August 12th flies had practically ceased to hatch out, and during the thirteen days the following were trapped from the heap:

Musca domestica L.	798
Stomoxys calcitrans L.	31
Chortophila cilicrura Rdi. ...	22
Fannia canicularis L.	4
Hydrotaea armipes F.	10

This experiment showed in a marked degree the effect of such attractive adjuncts as a bakery and numerous houses in close proximity to the manure bin. The flies did not hatch in great numbers from the trenches, the proportion of *M. domestica* being:

Direct from heap	682
From trenches	116

which gives a proportion of over 85 per cent. hatched direct from heap. The highest temperature recorded for this heap was about 50°. This difference in temperature does not however account for the difference in the number of the flies, as careful examination of Heap 1, before it was covered in, showed that it was not really infected, whilst the larvae could easily be seen in Heap 2.

Experiment III. Mixed material, principally horse and cow manure, was obtained from a neighbouring farm and placed on a trap at the laboratory prepared as already described. It was then well trodden down so as to represent the compacted form of storage. This was completed on June 29th. On July 1st the temperature at centre was 42°, on July 5th 55°, and on July 8th 58°. It subsequently fell on July 21st to 40°, but rose again two degrees on July 26th. The heap was covered in on July 12th, and remained closed till August 5th. During that time the following flies were trapped therefrom:

Eristalis tenax L.	8
Stomoxys calcitrans L.	8
Sargus cuprarius L.	15
Chrysomyza demandata F.	2
Fannia canicularis L.	6
Chortophila cilicrura Rdi.	9

There is a complete absence of *M. domestica*, the material having failed to become infected either at the laboratory or at the farm whence it was obtained. Most of the flies came from the heap direct.

Experiment IV. A heap of material similar to that used in Experiment III was established on June 29th at the laboratory. The manure was however in this case laid light without any treading. On July 1st the temperature was 57°, on the 5th 60°, on the 8th 38°, on the 21st 26°, and on the 26th 35°. The heap was covered in on July 12th, and remained covered till August 5th, during which period the following flies were obtained:

Eristalis tenax L.	13
Stomoxys calcitrans L.	9
Chortophila cilicrura Rdi.	14
Scatophaga stercoraria L.	4
Sargus cuprarius L.	2
Fannia canicularis L.	3

This heap produced 45 flies as against the 48 in Experiment III. All but three came from the heap direct. The total absence of *M. domestica* is again noticeable.

Experiment V. An experimental heap was established near the farm buildings belonging to the Rothamsted Experimental Station, and over a mile from the laboratory. The position of this and other heaps referred to as "at the farm" scarcely requires illustration. With the exception of two small cottages some 70 yards distant, there are no dwellings near these buildings, which include a stable for horses having behind it a large manure shed in which manure is stored in large quantities. The heaps used in this and other experiments at the farm were trenched and enclosed precisely as already described, and were placed some 20 yards from the stable door. Upon Heap 4 was thrown a barrow load of fresh horse manure every day, taken direct from the stable adjoining. The heap was started on June 28th and covered in on July 14th, and remained covered till August 4th. From it there hatched out only 26 flies, of which 16 were *Calliphora erythrocephala*, 5 *Chortophila cilicrura*, and 3 *Fannia canicularis*. Two others were small flies of undetermined species. No examples of *M. domestica* were obtained. The preponderance of "Blue bottles" is rather remarkable, and may have been due to a dead vole or some other small animal having got into the heap. Half of them came from the trench traps.

The highest temperature recorded for this heap was about 50° though it was probably higher at some time during my temporary absence owing to a slight accident.

Experiment VI. Close to the heap used for Experiment V some compacted mixed manure was trenched and enclosed as before. This heap was covered in about July 14th after being open to infection for 15 days. It remained covered till August 9th. The highest temperature recorded was 50°. During the period named the following 57 flies were obtained:

Eristalis tenax L.	3
Stomoxys calcitrans L.	2
Sargus cuprarius L.	10
Hydrotaea armipes F.	24
Fannia canicularis L.	17
Cordylurid Sp.?	1

Of the above only two came from the trench, both being *E. tenax*.

Experiment VII. A heap corresponding to that used in Experiment VI was made near the farm buildings. Mixed manure was laid on it light instead of compacted. The heap was enclosed about July 14th after being open to infection for 15 days, and remained closed till August 12th. From this heap the following flies emerged:

Ophyra leucostoma W.	36
Stomoxys calcitrans L.	4
Sargus cuprarius L.	13
Chortophila cilicrura Rdi.	37
Chrysomyza demandata F.	6
Fannia canicularis L.	3

The heap thus produced about 100 flies, all except one coming from the heap direct. It is remarkable that it produced nearly double the number of flies obtained from that used in Experiment VI, and it might be supposed that the loose laying of the manure had affected the figures. This supposition is not however supported by comparison with Experiments III and IV.

Experiment VIII. A small heap of garden and kitchen refuse behind the laboratory was covered over with a packing case and a trap fixed thereon. The refuse contained potato peelings, pea and bean pods, leaves, scrapings of a small hen coop, etc. The refuse was covered over on July 28th and remained closed till August 11th. During this period the following flies were trapped:

Muscina stabulans Fln. ...	...	13
Fannia canicularis L. ...	...	17
Chortophila cilicrura Rdi.	...	13
Ophyra leucostoma W. ...	...	4

This experiment was intended to test garden refuse as a breeding place for *M. domestica* under the conditions obtaining at the laboratory, and also in the hope of breeding *Musca autumnalis* De G.[1], which species was fairly abundant in the garden. Neither species emerged from the material.

Experiment IX. There is behind the farm buildings mentioned in Experiment V a large manure shed, the floor of which is cemented forming a kind of tank about 12 inches deep, and having an area of about 660 square feet. Here there was an accumulation of manure and straw taken from the stable adjoining, the sweepings from the stable being thrown on to it daily. It therefore contained both old and fresh manure, and an area of some 25 square feet was covered over with a tent of cheese cloth having a trap on the top. The lower edge of the tent was not fixed close to the cement but was heaped up with straw all round. Doubtless a good many flies escaped round the bottom. On the other hand many were caught and these proved to be nearly all *Stomoxys calcitrans* of which some 453 were taken in the ten days from August 9th to August 19th. Had the whole area of the manure yard produced flies at this rate, the total output would have been nearly 1200 per day, though a lower output is indicated by Experiment XVI. During the period only three *M. domestica* were taken together with a few *Anthomyidae*. Towards the end of this period *M. domestica* became more numerous about the stable.

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[1] Formerly known as *Musca corvina*.

Experiment X. Heap 1 was re-opened and the contained horse manure exposed for about a week. After closing in again no flies of any kind emerged.

Experiment XI. Heap 2 containing mixed manure was re-exposed and afterwards trapped. No flies were obtained.

Experiment XII. Heap 6 containing mixed manure was submitted to a second infection period but produced no flies.

Experiment XIII. Heap 3 was refilled with horse manure obtained from a large stud stable in the neighbourhood. This establishment is right away from dwellings, and precautions are regularly taken to disinfect the manure. That supplied for the experiment had not been treated in any way. The heap was made up on August 24th. On the 25th numbers of small maggots were observed on the surface, and these were evidently suffering from the heat. The temperature on the surface exactly where the maggots were wriggling was found to be 42°. Two inches below the surface it was 57°, whilst the centre of the heap gave 65°. A few *M. domestica* were observed inspecting the heap. Towards evening numbers of the maggots were found to have been killed by the heat. On August 30th the heap was completely enclosed. By September 3rd one *M. domestica* and several small *Anthomyidae* had appeared in the top trap

The traps of this heap were cleared on September 6th, when four *M. domestica* were taken. The experiment continued till September 10th, the total flies taken being:

M. domestica L. ...	... ...	12
Limnophora septemnotata Ztt. ...		37

Experiment XIV. Heap 1 at farm having been cleared out, fresh horse manure from the adjoining stable was placed in it in such quantities as were available from day to day. The heap was finally closed up on August 18th, having been open to infection for some 12 days. By August 24th no flies of any kind had emerged. By September 7th no *M. domestica* had been produced, but 18 *Fannia canicularis* were taken from the top trap and five from the sides. The experiment was continued till September 10th, by which date the following flies had been taken:

Stomoxys calcitrans L. ...	...	3
Fannia canicularis L. ...	...	14
Phaonia querceti Bouché	...	2
Chortophila cilicrura Rdi.	...	17
Calliphora erythrocephala Mg. ...		1

Experiment XV. On August 24th Heap 5 was again started with fresh horse manure from the adjoining stable. On August 30th the heap was closed in. Some of the manure in this heap had been purposely exposed for five or six hours close to the door of the stable where several *M. domestica* could be seen flying about. On September 3rd the traps of this heap were cleared and found to contain 6 *M. domestica*. Cleared again on September 7th, 5 were taken. Up to September 10th no more flies emerged. Two *Calliphora erythrocephala* were found in the side traps.

Experiment XVI. A wooden trap covering an area of about 9 square feet was placed in various positions on the permanent manure yard at the farm for 2 or 3 days at a time. In several cases no flies of any sort were caught, but in one position, at date about August 30th, it captured 16 *M. domestica*, 2 *S. calcitrans*, and 1 *F. canicularis*. Allowed to remain in the same place for several days longer no further flies were obtained.

Experiment XVII. A quantity of pure horse manure which had been sent to the laboratory for experimental purposes other than in connection with flies was found to have become infected in two or three days and contained many larvae of *M. domestica*. This material, in quantity about a bucketful, was covered over with a box trap. Examined from time to time the larvae apparently thrived but they all disappeared by the beginning of September, and by September 10th no flies had emerged. The slow development was doubtless due to the perfectly cold conditions owing to the small quantity of manure.

Experiment XVIII. At a cottage near the farm buildings it was found that the kitchen and garden refuse were placed in a pit in the ground some 20 or 30 yards from the house. When examined this pit was sodden with water and very offensive. Great numbers of flies were buzzing about it, though *M. domestica* was not in evidence. *L. caesar* was perhaps the commonest fly observed. A box trap was placed over a part of this rubbish pit and flies taken from time to time. In about a week some 78 flies were captured but only 1 example of *M. domestica* was included. The totals were as follows:

Musca domestica L.	1
Hydrotaea dentipes F.	22
Eristalis tenax L.	3
Eristalis arbustorum L.	4
Syritta pipiens L.	11
Ophyra leucostoma W.	13

Chortophila cilicrura Rdi. ...	14
Hydrotaea armipes F.	6
Spp. ?	4

To those who have kept in touch with much of the work recently carried out in connection with the fly problem, perhaps the most striking feature of the foregoing results will appear to be the small number of flies obtained from the experimental heaps. Having some two dozen traps in constant use I fully expected that some assistance would certainly be required in the mere mechanical work of counting and sorting the flies taken.

Thus in Dr Gordon Hewitt's experiments in Canada in 1913[1] a cubic yard of untreated manure used as a control experiment produced 13,332 flies. Large as this number is it appears almost trifling when compared with the figures given by Messrs Cook, Hutchison and Scales[2]. In their Table V, control heaps consisting of 4 bushels of manure (5 cubic feet) are cited as having contained 342,771, 385,403 and 273,520 pupae respectively. Whether such numbers are liable to occur in this country I have no records to enable me to decide, though doubtless numbers comparable to these might occur in say a crowded city area. The most heavily infected manure I was able to obtain, in quantity about 14 cwt. (see Experiment II), produced only some 865 flies, of which 798 were *M. domestica*. The wet summer of 1915 may perhaps account to some extent for the results obtained, though Experiment II was carried out in comparatively fine weather. The flies obtained in this experiment were however quite sufficiently numerous to emphasize the admitted danger of uncontrolled manure heaps in close proximity to dwellings, and we may at once turn to the consideration of the results as applied to heaps under more rural conditions.

Experiments I, III and IV, with horse and mixed manures, produced only three examples of *M. domestica*. During the early part of the season this fly was by no means common, and even later in the summer it was not found to occur in great numbers in or near the laboratory. It was not obtained from garden refuse. A small quantity of horse manure (Experiment XVII) became rather heavily infected at the laboratory though this was undoubtedly due to its having stood for some days actually in the laboratory, and a few flies first attracted into the rooms had soon found the material so conveniently at hand.

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[1] *Journal of Economic Entomology*, vol. VII. No. 3, p. 281 *et seq.* 1914.

[2] *U.S. Dept. of Agriculture, Bul.* 245, July 1915.

In Experiment XIII in which the heap was enclosed on August 30th there was every opportunity for *M. domestica* to breed. The heap was continually watched before covering in and it is certain that *M. domestica* did not visit it in great numbers. The small maggots destroyed by the heat were probably those of the *Limnophora*, and in any case the heat would not account for the small number of *M. domestica* since similar conditions obtained in Experiment II. The results obtained with heaps at the farm buildings were more interesting. The first three heaps produced no examples of *M. domestica*, nor was it an easy matter to find this species in or about the stables. Later on the fly appeared in some numbers and random catches with the net gave the following:

Musca domestica L.	44·4 %
Musca autumnalis De G. ...	31·48 %
Muscina stabulans Fln.	24·08 %

To these figures, however, I cannot attach very great importance since from Experiment IX it would seem that *Stomoxys calcitrans* should have been the commonest fly, whereas none were taken in the net. On August 12th I inspected adhesive fly-papers in one of the cottages already referred to as near the farm buildings. One of these papers, stated to have been in use about a fortnight, had caught some 300 flies, almost all *M. domestica*. Another paper in use for the same period contained perhaps a few more. Careful search failed to disclose any special nidus for these flies. The most likely place seemed to be the garbage pit referred to in Experiment XVIII. Although flies of many species were obtained from this pit only one proved to be *M. domestica*. From other experiments it may be assumed that a few of the flies taken on the fly-papers came from the permanent manure yard at the farm buildings. There is nevertheless a strong temptation to suppose that with the well-known tendency of the species to enter houses, the cottages in question formed an attraction for every wandering fly in the neighbourhood and they were thus more likely to be found there than elsewhere. Heap 4 having produced no house-flies from the first filling, a second supply of fresh horse manure was trapped but without producing *M. domestica*. Heap 5 treated in the same way produced altogether 11 of this species. Meanwhile endeavours had been made to find the fly breeding in the large permanent heap already referred to. In Experiment IX 3 *M. domestica* were taken together with about 453 *Stomoxys calcitrans*, whilst from another part of the same heap (Experiment XVI) 16 specimens of the former were secured. The latter experiment furnishes the only real evidence that *M. domestica* was breeding at all in this heap, and moving the trap on to other areas did not secure any further examples.

I see no reason to regard these experiments as furnishing results other than typical of the conditions under which they were conducted, and in the absence of further evidence it would seem that the following conclusions may be drawn.

That whilst, as already fully recognised, the house-fly is liable to breed in large numbers in stable refuse which is stored in close proximity to dwellings, the governing factor is found in the dwellings rather than in the manure heap, the latter merely serving as a secondary convenience, providing a breeding place for the flies which have been attracted to the houses in search of food.

That the open farm manure heap *far away* from houses is but little frequented by house-flies, and then only later in the season when the insect has become numerous and widely dispersed.

That the spent manure heap, in which fermentation has practically ceased, produces under rural conditions at least practically no flies at all.

That although the farm heap may produce but few house-flies, it is a prolific source of *Stomoxys calcitrans*, and those agriculturists who value the comfort and health of their animals should treat all manure with a view to the destruction of the larvae of this pest.

It should be clearly understood that the above conclusions apply to manure heaps far distant from houses. Where the farm dwelling and the farm buildings adjoin, as they do in so many cases, the danger of the manure heap becomes much greater, particularly where dairies or other food-preparing departments are in proximity to farm refuse.

For the town manure heap, under which category I include that from which the material used in Experiment II was obtained, no regulations can be too drastic, and it is but little creditable to our local authorities, and even less so to the proprietors, that such conditions should be permitted to exist.

Mention has been made of *Stomoxys calcitrans* as a pest to cattle. The "biting house-fly," as it has been called, is a blood-sucking insect possessing great capabilities as a carrier of disease, and it is by no means inclined to distinguish for alimentary purposes between the human and the equine species. There is however another fly, which on account of its numbers and persistency, is probably a far greater nuisance. I refer to *Musca autumnalis* De G. Swarming in the open, it enters houses somewhat less readily than *M. domestica*, though after *Fannia canicularis* it is perhaps our next most frequent uninvited guest. In autumn it is given to entering houses, especially attics and disused apartments, in enormous numbers, and so-called hibernating house-flies are almost invariably of this species. Professor Poulton has recorded them[1], in his house in the Isle of Wight, in such numbers as to contaminate the secondary water supply. It is not remarkable that the species has received such slight mention in current works on the house-fly since it is only with some practice that it can be distinguished therefrom. The early stages of the species have not so far as I am aware been observed in this country.

I succeeded in breeding the fly from bullock dung. A sample of this material sent to the laboratory for experimental purposes contained numerous bright yellow larvae, some of which I preserved, others being kept till they matured. Having found that *M. autumnalis* De G. resulted from these larvae I inspected bullock dung in the fields and had little difficulty in finding the larvae again. It is probable that this is by no means the only material in which the species may be found though it is evident that it is one of the substances in which it regularly breeds. I hope shortly to collect existing records of this fly and to publish fuller details of my own observations on its life-history.

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[1] *Proc. Ent. Soc. Lond.* p. xxi–xxii, 1915.

(*Received October* 14*th*, 1915.)

STUDIES OF A SCOTTISH DRIFT SOIL.

PART I. THE COMPOSITION OF THE SOIL AND OF THE MINERAL PARTICLES WHICH COMPOSE IT.

By JAMES HENDRICK, B.Sc.,
AND WILLIAM G. OGG, M.A., B.Sc.

(Department of Agricultural Research, University of Aberdeen.)

Most of the soil investigations in this country have been conducted at Rothamsted or other parts of South Britain and the soils examined have been chiefly those overlying the stratified rocks of the southern half of England. These, however, are by no means typical of the whole of Britain and research is needed into soils found extensively in Scotland and other parts of Britain which differ greatly in their origin, nature and properties from those which have hitherto been the chief subjects to which British investigators have devoted attention. The various agricultural colleges are taking up the study of the soils of their respective districts, and when the Farm of Craibstone was acquired as an Experiment Station by the North of Scotland College, a series of soil investigations was immediately commenced.

Craibstone is situated about six miles north-west of Aberdeen and is a farm typical of much of the agricultural land of the district and of the North of Scotland generally. The soil is a boulder clay overlying granite and varies much in depth; the subsoil also varies much in depth and texture, passing within a short distance from sand and gravel to clay. The underlying rocks in Aberdeenshire are chiefly granites and metamorphic rocks, and great parts of the neighbouring counties are founded on rocks of a similar nature.

More important, however, than the fundamental formation is the drift material by which it has been overlaid during the ice age and from which most of the soils have been derived. This drift differs much in different parts of Britain. In places it attains a thickness of over two hundred feet, and quite near may be areas from which it is absent altogether.' In some parts it consists of the old soils and subsoils of the original land surface; in others of the ground-down portions of igneous, metamorphic, and very ancient stratified rocks, and between these extremities there are all intermediate stages. Thus the drift of the South-east of England - the Clay-with-Flints, for example—is very different from the drift of the North of Scotland, the one being derived from the old surface soil and late formations, while the other is largely the product of the granitic and metamorphic rocks of the Scottish Highlands. Soils of this latter type cover a great part of North Britain, and Craibstone furnishes a typical specimen of the Northern Drift Soil.

General composition of the material.

The sample used in these investigations was obtained from the South Meethill Field at Craibstone which is being used for field experiments, and in which lysimeters have been built to study the drainage.

The sample used was taken to a depth of nine inches, air dried, and freed from large stones.

The methods of analysis (mechanical and chemical) were, except where otherwise indicated, those adopted by the members of the Agricultural Education Association.

The calcium carbonate present and the "lime requirement" were determined by the methods of Hutchinson and MacLennan[1].

From the mechanical composition it will be seen that the soil under investigation is a coarse sandy soil comparatively rich in organic matter and poor in clay. In determining clay, the liquid was evaporated in bulk and therefore much organic matter dissolved by ammonia was present and was estimated as clay "dried at 100° C.," but even with this included, the total was less than 9 per cent. That this is a great overestimate is shown by the fact that after ignition the weight, *i.e.* the ignited mineral matter of the clay, is less than 4 per cent. of the dry soil.

The chemical analysis shows the soil to be rich in phosphoric acid and potash both "total" and "available."

It contains no carbonate of lime, and has a high "lime requirement," but in spite of this, good crops have been grown for many years without the application of lime.

[1] *Journ. of Agric. Sc.* 1914, 6, 323-327; 1915. 7, 75-105.

TABLE I. *Mechanical Analysis.* Dried at 100° C.

	Approx. diam. in millimetres	Dried at 100° C.	After ignition
		per cent.	per cent.
Fine gravel	3–1	10·09	9·93
Coarse sand	1–·2	30·08	29·73
Fine sand	·2 –·04	26·20	25·80
Silt	·04 –·01	14·18	12·47
Fine silt	·01 –·002	9·62	7·63
Clay	·002–0	8·88	3·80
Total of above	—	99·05	89·36
Loss on ignition	—	—	9·69
Dissolved (by difference) ...	—	—	0·95

TABLE II. *Chemical Analysis.* Fine earth dried at 100° C.

	By treatment with strong hydrochloric acid	Soluble in 1 per cent. citric acid (Dyer's method)
	per cent.	per cent.
Sand and insoluble silicates ...	88·81	—
Phosphoric acid	0·36	0·092
Potash	0·49	0·033
Lime	0·53	0·141
Magnesia	0·19	0·031

Loss on ignition (humus, etc.) ...	9·54
Nitrogen	0·30
Lime as carbonate	nil
"Lime requirement" as $CaCO_3$ ...	0·236

The soils of North Wales have been examined by Robinson[1], and certain of them appear to resemble in many respects those of Scotland. Among other things he found carbonate of lime to be absent from most of the soils examined. This entire absence of carbonate of lime from fertile soils is noteworthy since it has been looked on by many agricultural writers as an essential constituent, and according to Russell[2] "calcium carbonate is often present in small amounts only, but it plays a controlling part in soil fertility."

Chemical composition of the soil fractions.

These preliminary determinations of the general composition of the soil do not throw much light on its history or on the origin of the characteristics which distinguish soils of this type from, for instance, those of the South of England soils which are also of glacial origin.

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[1] *Journ. of the Board of Agr.* 1915, **22**, 3.

[2] *Soil Conditions and Plant Growth* (New Edition), 1915, p. 63.

Differences in origin and method of formation give rise to differences both in physical structure and in chemical composition. The differences in physical structure are measured, though only in a crude way, by mechanical analysis, by which the particles of different sizes are separated into arbitrary groups. Chemical composition is usually determined by treatment with a conventional strength of acid. For certain purposes these determinations are sufficient, but for a close comparison of soil types much more is needed. Even complete mineral analysis of the whole soil by fusion or treatment with hydrofluoric acid does not help us much. We have endeavoured to gain further information as to the origin and constitution of this soil by subjecting the different mechanical fractions to ultimate analysis and comparing the results with those obtained elsewhere by others.

Analysis of the fractions of Craibstone soil.

A portion of the prepared sample was fractionated by the ordinary method of mechanical analysis, and the fractions, after ignition, analysed by fusion methods. The results are expressed in Table III as percentages of the relative fractions, and in Table IV as percentages of the total soil.

TABLE III. *Ultimate analysis of mechanical fractions.*

(Calculated as percentages of dry mineral matter of fractions.)

	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	Na_2O	P_2O_5
Fine gravel ...	84·96	8·56	1·10	0·88	0·36	1·49	1·58	0·07
Coarse sand ...	83·92	9·34	1·12	1·79	0·38	1·78	1·21	0·08
Fine sand ...	73·87	13·47	4·21	3·05	1·05	1·73	1·53	0·12
Silt	70·15	14·04	5·86	2·15	1·06	1·48	3·89	0·21
Fine silt ...	67·21	18·91	7·85	1·45	1·63	2·51	1·27	0·29
Clay	44·08	27·64	21·81	0·58	1·61	1·10	0·96	0·36

TABLE IV. *Ultimate analysis of mechanical fractions.*

(Calculated as percentages of total dry soil.)

	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	Na_2O	P_2O_5	Total
Fine gravel	8·44	0·85	0·11	0·09	0·04	0·15	0·16	0·01	9·85
Coarse sand	24·96	2·78	0·33	0·53	0·11	0·53	0·36	0·02	29·62
Fine sand	19·05	3·48	1·09	0·79	0·27	0·45	0·39	0·03	25·55
Silt	8·75	1·75	0·73	0·27	0·13	0·18	0·49	0·03	12·33
Fine silt	5·13	1·44	0·60	0·11	0·12	0·19	0·10	0·02	7·71
Clay	1·68	1·05	0·83	0·02	0·06	0·04	0·04	0·01	3·73
Total	68·01	11·35	3·69	1·81	0·73	1·54	1·54	0·12	88·79

In considering the figures in Tables III and IV it is to be remembered that the method of mechanical analysis adopted involves the treatment of the soil with dilute hydrochloric acid before fractionation is performed, and that therefore all compounds easily soluble in acid were removed and are not shown in the analyses. Table II shows that appreciable quantities of phosphoric acid, potash, lime, and magnesia were dissolved from the original soil by dilute citric acid. No doubt quantities of all these as well as of iron and alumina were dissolved by the dilute hydrochloric acid used before the mechanical separation into fractions was commenced. In the case of phosphoric acid much the greater part of this constituent present in the soil is easily soluble in dilute hydrochloric acid, for whereas Table II shows that the soil contained 0·36 per cent. of phosphoric acid soluble by digestion in strong hydrochloric acid, Table IV shows that only 0·12 per cent. was found in the whole of the mechanical fractions.

Table III shows that, as has been found by all previous investigators of this subject, the percentage of iron and alumina increases and the percentage of silica decreases with a decrease in the size of the particles. In the case of other elements the results of different investigators do not agree so well, but the majority of workers found an increase of potash, soda, lime, magnesia, and phosphoric acid in the smaller particles. The evidence with regard to some of these is, however, very conflicting.

Puchner[1] working on three different soils a heavy loam, a silty soil (loessial) and a coarse sandy soil from gneiss —found the content of lime, magnesia, and phosphoric acid to be irregular, and that of silicic acid, soda, and potash to be smaller in the finer fractions.

Schneider[2], for a residual soil from the disintegration of augite-andesite, found that the percentage of lime decreased with the size of the particles, while Loughridge[3] found the lime irregular, and Clerk, Gortner and Vail[4] found the highest percentage of this constituent in the silts, and the lowest in the clays.

Schneider[5] found the percentages of magnesia and phosphoric acid irregular.

In Craibstone soil the percentage of phosphoric acid in the fractions increases regularly as the size of the particles becomes less. This is in agreement with the results of most workers. It indicates that there is a greater percentage of phosphoric acid present in highly insoluble forms in the finer particles of the soil; but it is to be remembered that, as has been shown above, the greater part of the phosphoric acid of the soil was removed during the preliminary treatment with dilute hydrochloric acid before the mechanical fractions were separated.

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[1] *Landw. Vers.-Stat.* 1907, **66**, 463

[2] *Amer Jour. Sci.* 1888, **36**, 236.

[3] *Amer. Jour. Sci.* 1874, **7**, 17.

[4] *Amer. Chem. Jour.* 1908, **39**, 163.

[5] *loc. cit.*

Lime, on the other hand, shows a maximum percentage in the fine sand and falls to a minimum in the clay, while potash and soda are irregular, but both show their smallest percentages in the clay. A similar fall in the case of lime was found by Schneider in the case of the clay from the residual soil from augite-andesite already referred to. This soil was derived from the Rockland Ridge, Washington. On the other hand most investigators have found the highest percentages of potash and lime in the finest fractions.

Table IV shows the distribution in the different mechanical fractions of the constituents calculated as percentages of the total dry soil. The interest of this table lies in the fact that it brings out clearly how large a store of potential food material is present in the coarser fractions which constitute so large a proportion of Craibstone soil. For instance in the case of lime the greatest proportion is present in the fine sand, and over two-thirds of the total lime and nearly two-thirds of the total potash are present in the coarse sand and fine sand taken together. On the other hand the clay contains only a very minute proportion of either the potash or lime of this soil. Robinson (*loc. cit.*) considers the richness in potash of the soils of North Wales due to the potash minerals in the fractions usually classed as sand and gravel.

The connection between the nature and origin of a soil and the composition of its mechanical fractions.

While in general the percentage of silica is found to decrease and the other elements to increase in the finer fractions, the relative decreases and increases differ very much in different soils. These variations are connected with differences in the nature of the soils and in the conditions under which they were formed and at present exist.

There have been at least three processes at work which influence the composition of the fractions, and may lead to differences in soils which were in origin alike.

(*a*) *Mechanical grinding.* In the grinding down of rocks in the process of soil formation, whether by glacial action or by water, the soft materials are most readily powdered and consequently there is a large proportion of these in the finest fractions.

(*b*) *Action of solvents.* Certain substances are much more readily dissolved than others, *e.g.* in granite the felspars are more readily attacked than quartz. This again leads to some minerals occurring more frequently than others in certain fractions.

(*c*) *Absorption and redeposition of dissolved matter* on the surface of the soil particles takes place through changes of temperature and concentration, and other changes affecting the conditions existing in the soil solution.

The composition of all the fractions of a soil will be influenced by the extent to which these three processes have been carried during the soil formation. Though these processes all operate to some extent in practically every case and produce that rough general similarity in chemical composition which is found to exist in particles of similar grade from widely different soils, sometimes one process predominates and sometimes another and hence considerable differences arise in all grades of particles between soils of different history. For example, where solvent action has not proceeded far and the weathering is chiefly mechanical, the fractions will differ in chemical composition from those of soils which have long been subjected to the action of solvents. Even mechanical forces give rise to different types of chemical composition in similar mechanical separates, for the mechanical action of water is more selective than that of ice, and, other things being equal, there would tend to be a greater proportion of hard materials in the silts and clays of glacial origin than in those due to attrition by running water.

The effect of differences in nature and origin on the chemical composition of the separates was drawn attention to by Dumont[1]. He showed that two soils containing about the same proportion of potash (0·894 and 0·853 per cent. respectively) had very different percentages in their mechanical fractions. In one, a fine grained soil from an experimental field at Grignon, the coarse sand contained 0·864 per cent. of potash, the fine sand 0·992 per cent., and the clay 0·940 per cent.: while in the other, a coarse sandy granitic soil from Creuse, the coarse sand contained 1·33 per cent., the fine sand 0·58 per cent., and the clay 0·51 per cent. When expressed as percentages of the whole soil, the difference in the distribution of the potash is even more striking, for the soil from Grignon contained 16·8 per cent. of clay and 17·2 per cent. of coarse sand as compared with 4·5 per cent. of clay and 44 per cent. of coarse sand in that from Creuse.

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[1] *Comptes Rendus*, 1904, **138**, 215–217

Failyer, Smith and Wade[1], of the U.S. Bureau of Soils, studied the separates of a large number of soils of the United States, and in their report on "The Mineral Composition of Soil Particles" they give a useful *résumé* of the whole subject. In their mechanical analyses they divided their samples into three grades only, namely, sand (2–·05 mm.), silt (·05–·005 mm.), and clay (·005–0 mm.). Phosphoric acid, potash, lime, and magnesia were determined in these fractions by fusion methods.

One of the groups of soils thus examined came from the Coastal Plains "which are made up of unconsolidated gravels, sands, silts and clays, derived in most part from the erosion of the Piedmont Plateau and other inland areas. These materials were mainly deposited on the then ocean floor and have been brought to their present level by the elevation of the land areas....While the soils of this region present many diversities among themselves, due to special circumstances affecting their deposition or subsequent history, they all differ much from the parent rock and have been subjected to excessive weathering and leaching." Seven soils of this group were examined.

A second group consisted of residual soils from crystalline and metamorphic rocks. "The method of formation, in addition to pulverisation, has been one of removal of certain parts of the rocks, either by solution or mechanically by moving water or air, leaving the present soil as a residue. The material forming the soil may differ but little chemically and mineralogically from the rocks whose breaking down has produced the soil, or it may depart much from them." Only three soils of this group were examined.

A third class examined consisted of soils of glacial origin. This group "includes soils formed from material deposited by glaciers or this material somewhat reworked by water, and also loessial soils, consisting largely of particles the size of silt, which have been carried from other glacial areas and deposited over the underlying material....The glacial soils consist largely of crushed rocks. Much of the material composing them has not been profoundly weathered. They are therefore quite similar in composition to the residual soils, and hence differ from those of the Coastal Plains." Ten soils of this class were examined.

The soils of each class examined by Failyer, Smith and Wade differ considerably from each other, but show certain general similarities among those of the same class. Each class exhibits distinct differences from the other classes. In particular the soils of the Coastal

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[1] *U.S. Bureau of Soils Bull.* **54**, 1908. The Mineral Composition of Soil Particles.

TABLE V. *Chemical analysis of fractions of American soils.*

(Calculated as percentages of the dry mineral matter.)

	Approx. diam. in millimetres	CaO			MgO			K_2O			P_2O_5		
		Coastal plain	Glacial	Residual	Coastal plain	Glacial	Residual	Coastal plain	Glacial	Residual	Coastal plain	Glacial	Residual
Sand ...	2–·05	·07	1·24	·50	·09	·54	·48	·37	1·72	1·60	·03	·15	·07
Silt ...	·05 –·005	·19	1·30	82	·14	·88	·86	1·34	2·35	2·37	·10	·23	·22
Clay ...	·005–0	·55	2·69	·94	·61	1·80	1·24	1·76	3·08	2·86	·34	·86	·67

TABLE VI. *Comparison of Craibstone and South of England soils.*

(Chemical analysis of mechanical fractions, calculated as percentages of dry mineral matter.)

	SiO_2		Al_2O_3		Fe_2O_3		CaO		MgO		K_2O		P_2O_5	
	Craib-stone	English	Craib-stone	English	Craib-stone	English	Craib-stone	English	Craib-stone	English	Craib-stone	English	Craib stone	English
Fine gravel	84·96	94·4	8·56	3·0	1·10	2·1	0·88	0·4	0·36	0·8	1·49	0·6	0·07	0·06
Coarse sand	83·92	93·9	9·34	1·6	1·12	1·2	1·79	0·4	0·38	0·5	1·78	0·8	0·08	0·05
Fine sand	73·87	94·0	13·47	2·0	4·21	1·2	3·05	0·4	1·05	0·04	1·73	1·5	0·12	0·02
Silt	70·15	89·4	14·04	5·1	5·86	1·5	2·15	0·8	1·06	0·3	1·48	2·3	0·21	0·03
Fine silt	67·21	84·1	18·91	7·2	7·85	2·6	1·45	1·1	1·63	0·2	2·51	3·2	0·29	0·1 (*a*)
		64·3		19·3		7·6		2·2		0·4		5·3		0·4 (*b*)
Clay	44·08	53·2	27·64	21·2	21·81	13·2	0·58	1·6	1·61	1·0	1·10	4·9	0·36	0·4 (*c*)
		49·0		29·8		13·1		1·5		1·0		3·4		0·7 (*d*)

Plains differ greatly as a class from the Residual and Glacial Soils. These differences are dealt with in detail in the original memoir. In order to illustrate the general effects on the composition of their differences in origin we have calculated the average composition of the separates of the three groups of soils, and the results are shown in Table V.

This Table is not given in the original bulletin of Failyer, Smith and Wade, but has been calculated from the figures given in their detailed tables.

Summarising their work, Failyer, Smith and Wade point out:

1. That "as a general rule, the smaller particles of soils are richer in potassium, calcium, magnesium, and phosphorus than the larger particles."

2. That "the concentration of these elements in the finer components is the more pronounced as the soils have undergone more extreme weathering."

3. That "in glacial soils and others resulting largely from mechanical processes, the coarser particles are relatively high in the percentages of potash, lime and magnesia."

Comparing British soils in the same way we find a somewhat similar contrast in the composition of the fractions according to the origin of the soil. A number of English soils from the gault, bargate, brick earth, and clay with flints formations were fractionated and the fractions were analysed by Hall and Russell[1]. The average of these[2] may be taken to represent the much weathered and decomposed minerals of the soil of the South of England as contrasted with the granitic and metamorphic glacial drift of the north-east of Scotland, which though pulverised by glacial action has not undergone the age-long weathering processes of the southern English soils.

In Table VI we have placed side by side our analyses of the fractions of Craibstone soil and the average of the analyses of similar fractions of English soils in order to illustrate the striking differences between the constitution of these two classes of soils. Hall and Russell divide the "fine silt" in their analyses into two parts, shown as (*a*) and (*b*) in Table VI. (*a*) consists of particles from ·01 to ·005 mm. in diameter, while (*b*) consists of particles from ·005 to ·002 mm. in diameter. They also give two sets of figures for "clay," shown in Table VI as (*c*) and (*d*). Under (*c*) is given the analysis of clay from

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[1] *Jour. Agri. Sci.* 1911, **4**, 181–223.

[2] Russell, *Soil Conditions and Plant Growth*, 1915, p. 54.

"fertile soils" while under (*d*) is given the analysis from "less fertile soils."

Table VI shows:

(1) That the percentage of silica is smaller in all the fractions in Craibstone soil than in the corresponding fractions of the English soils. In the case of the three coarser fractions of English soil 94 per cent. or over consists of silica. That is, these fractions almost entirely consist of particles of more or less finely powdered silica. Even in the silt about 90 per cent. is silica, and the coarser part of the fine silt contains almost as great a percentage of silica as the fine gravel of Craibstone soil. The granite of the Aberdeen neighbourhood in the unweathered condition contains about 70 per cent. of silica and 18 per cent. of alumina. We may conclude, therefore, from the analyses that the coarser fractions of Craibstone soil contain much unweathered or partially weathered granitic material in addition to silica. This conclusion was confirmed by the microscopic examination of these coarser fractions and by comparison of them under the microscope with similar fractions separated from powdered granite. The granite used for these comparisons was obtained from a local quarry.

(2) The coarser fractions of Craibstone soil are much richer in alumina than the corresponding fractions of the English soils. It is only in the finest fractions, fine silt and clay, that the English soils are at all comparable in respect of alumina with Craibstone soil. To a certain extent the case of iron presents similar differences.

(3) The coarser fractions of Craibstone soil are much richer in potash and lime than the corresponding fractions of the English soils, but the finer fractions are poorer. A similar richness in potash of the coarse fractions was, as we have seen, noted by Robinson for soils of N. Wales. Whereas in the English soils the finest fractions, fine silt and clay, are the richest in potash and lime; in Craibstone soil the clay is the poorest of all the fractions in both potash and lime.

While Craibstone soil thus differs greatly from soils of the south-east of England, there is a general similarity in type, so far as we have data for comparison, between it and the American soils which have been produced by mechanical pulverisation rather than by profound chemical weathering. Thus Craibstone soil is much nearer in type to the Glacial or Residual soils of Table V than to the Coastal Plain soils. On the other hand the South of England soils conform more nearly in type to those of the Coastal Plain than to the Glacial or Residual soils.

CONCLUSIONS.

The general conclusions to be drawn from the chemical composition of the mechanical separates is that Craibstone soil, which may be taken as representative of a large class of glacial drift soils of the north of Scotland, is composed largely of particles which have not undergone profound chemical weathering, but consists of the original granitic minerals mechanically ground with only comparatively superficial chemical alteration.

The coarser particles which form so large a part of this soil contain great stores of lime in particular, and also of other bases such as potash, soda, and magnesia.

There is a wide difference between such a soil as that of Craibstone and soils of the south-east of England, for instance, which are composed of materials which have been subjected to age-long chemical weathering. When, as at Rothamsted, such soils are of glacial origin, they probably represent glacial detritus derived mainly from materials profoundly weathered long before the glacial period.

It is necessary, therefore, to examine carefully the whole circumstances and to exercise much caution before we apply to soils of Craibstone type conclusions arrived at either as to physical and chemical properties or manurial requirements by the study of the soils of the south-east of England.

We wish to express our indebtedness to Mr James Strachan, M.A., B.Sc., now in the Soudan, formerly of this Department, who made the chemical analyses of the mechanical fractions of Craibstone soil.

(*Received November* 15*th*, 1915.)

CAUSE AND PREVENTION OF RANCIDITY IN PALM NUT KERNEL CAKE.

By R. B. CALDER,
Gonville and Caius College.

(School of Agriculture, Cambridge.)

One of the most common complaints of users of palm nut kernel cake is that it is liable to become rancid on keeping. Rancidity developing in an oil-seed residue like palm nut kernel cake is likely to be due to splitting of the fats of the cake by a fat-splitting ferment or enzyme—a lipase—formed under certain conditions. The resting seeds do not contain lipase, but they are likely to contain a zymogen from which under suitable conditions lipase is formed. The lipase would then split the fats or oils with the formation of rancid-smelling fatty acids. According to Reynolds Green, lipases act most rapidly at 55° C. Their activity is slowed at 60° C. At 72° C. the lipase is destroyed, and, of course, its action ceases.

The following experiments show that rancidity in palm nut kernel cake is due to the formation of a lipase. A quantity of cake was finely ground, and portions placed in a number of bottles. Some were kept dry, others moistened with water. To the moistened samples a little toluene was added to prevent putrefaction. The bottles were well stoppered to prevent evaporation, heated in a water bath as described below, and afterwards kept at various temperatures in an incubator.

These experiments show that palm nut kernel cake when kept warm and moist for some time becomes rancid, but that it keeps well at the ordinary temperature if dry. The production of rancidity is prevented by heating for a long time to 60° C., or for a short time to 70° C. These facts are in accord with the conclusion that the rancidity is caused by the action of a lipase set free from a zymogen present in the seed. The important practical point is that rancidity is prevented by heating for a short time to 70° C.

Sample No.	Treatment	Result
1	Kept dry at ordinary temperature for 10 weeks.	No trace of rancidity.
2	Heated dry for 1 hour at 75° C., then kept for 10 weeks at room temperature.	No trace of rancidity.
3	Moistened and incubated at 25° C.	Rancid in 3 days.
4	Moistened and kept at room temperature.	Rancid in a few days.
5–8	Moistened, incubated at 22° C. for 24 hours to change zymogen to lipase. Then heated to 30° C ½ to 3 hours. Then replaced in incubator.	Rancid in a few days.
9–12	Same as 5–8, but heated at 40° C.	Rancid in a few days.
13–16	Same as 5–8, but heated at 50° C.	Rancid in a few days.
17–20	Same as 5–8, but heated at 60° C.	Sample heated only ½ hour, became rancid in a few days. Samples heated for 1 hour or longer remained sweet.
21–24	Same as 5–8, but heated at 65° C.	Half hour sample rancid, others remained sweet.
25–28	Same as 5–8, but heated at 70° C.	All samples remained sweet for 10 weeks.
29–32	Same as 5–8, but heated at 75° C.	All samples remained sweet for 10 weeks.
33–36	Same as 5–8, but heated at 80° C.	All samples remained sweet for 10 weeks.
37–40	Same as 5–8, but heated at 90° C.	No rancidity, but sample heated for 3 hours had a smell, possibly due to decomposition by long heating.

A second set of experiments was then carried out as follows. Some ground cake was kept warm and moist until it became rancid. It was then ground up with 5 per cent. common salt solution and incubated at 25° C. for 24 hours. The liquid part was then separated by filtration under pressure. It was a brown opalescent liquid with an acid reaction. It was divided into halves, one of which was boiled for 10 minutes.

Emulsions of castor oil, palm nut kernel oil, and coconut oil were made by means of water and gum arabic. Several tubes of each were treated with boiled and unboiled extract of rancid cake as prepared above. Each tube was exactly neutralised with sodium carbonate solution after addition of neutral litmus. All the tubes were then placed in the incubator at 25° C. After a few days all the tubes containing boiled extract were still neutral, whilst those containing unboiled extract had all become acid in 12 hours. This experiment shows that it is possible to dissolve the lipase out of rancid palm nut kernel cake. The lipase thus dissolved will turn other oils rancid if brought into contact with them under suitable conditions.

Finally boiled and unboiled extract were added to six samples of the cake which had been heated to 70° C. and incubated for some time without turning rancid. After a few days in the incubator all the samples (with one exception) to which unboiled extract had been added became rancid, whilst the samples mixed with boiled extract remained perfectly sweet. This experiment shows that extracted lipase can turn cake rancid.

To determine if rancidity is preventable by heating the dry powdered cake, samples were treated as follows:

Sample No.	Treatment	Result
1–3	Dry powdered cake heated at 30° C. for 1 hour, moistened, toluene added and placed in incubator at 28° C.	In less than a week all three samples became quite rancid.
4–7	Heated at 40° C. for 1 hour, otherwise treatment same as 1–3.	Ditto
8–11	Same, but heated at 50° C.	In a week, three quite rancid, one only slightly so. In 10 days the fourth sample also was quite rancid.
12–15	Same, but heated at 60° C.	Three quite sweet after 17 days, one had a slight trace of rancidity.
16–19	Same, but heated at 75° C.	No rancidity in any case after three weeks.
20–23	Same, but heated at 80° C.	Ditto
24–27	Same, but heated at 90° C.	No rancidity after a fortnight in three samples, but the fourth was slightly rancid.
28–32	Same, but heated at 100° C.	No rancidity in any case after a fortnight.

CONCLUSION.

Palm nut kernel cake, if kept dry and cool, remains sweet for at least 10 weeks. If kept moist and warm it becomes rancid in a few days. The cake contains a zymogen which under the influence of warmth and moisture forms a lipase. The lipase then turns the oil rancid. The lipase can be destroyed by heating the moistened cake to 70° C. for a short time. If the dry cake is heated the zymogen is usually destroyed, but dry heating is not so certain to destroy it as heating when moist.

(*Received December* 10*th*, 1915.)

THE FUNGICIDAL PROPERTIES OF CERTAIN SPRAY-FLUIDS.

BY J. VARGAS EYRE AND E. S. SALMON.

(*Research Department, South-Eastern Agricultural College, Wye, Kent.*)

INTRODUCTORY.

THE object of the experiments described below was to establish more clearly to what the fungicidal value of alkaline sulphide solutions is to be attributed.

For this purpose a study was made during 1914 and 1915 of the fungicidal action of certain chemicals, principally sulphides, on species of the "powdery mildews" (*Erysiphaceae*) under as exact conditions as possible. Information, based on carefully controlled experiments with actively-growing patches of the mildew, appears to be entirely lacking, notwithstanding the large amount of attention which has been given to the subject.

The economic importance of combating these mildews is very considerable. To take one instance—that of the American Gooseberry-mildew—it has to be recognised that the continuance of the commercial cultivation of this fruit depends upon a satisfactory spray being found. In this connection the lime-sulphur wash has proved, under practical conditions[1], of great value in protecting the gooseberry bush from early attacks of the mildew, but the strongly-adherent deposit produced by this wash renders its use objectionable for later sprayings on account of the disfigurement caused to the berries[2]. A solution of "liver-of-sulphur" leaves no visible deposit and has been commonly recommended against the American Gooseberry-mildew, but certain experiments carried out by one of us[1] in 1913 appeared to throw considerable doubt upon the efficacy of this material as a spray. Moreover, the frequently-observed scorching action on the foliage brought about by solutions of "liver-of-sulphur"[1, 2] make it desirable to find some material of fungicidal value which is harmless to foliage and does not mark the berries.

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[1] Salmon, E. S., in *Journ. South-Eastern Agric. Coll.* XXII. 403 (1913) [1914].

[2] *Idem, loc. cit.* p. 423.

The wash recommended almost universally as efficacious against the class of "powdery mildews" (*Erysiphaceae*) is a solution of "liver-of-sulphur"—generally understood to be a mixture of various sulphides of potassium, although latterly the less valuable sodium carbonate has replaced potassium carbonate in the preparation of "liver-of sulphur" for horticultural purposes. An examination of the literature on this subject, however, reveals the fact that definite information is lacking on the two most essential points, (1) the strength at which the solution is fungicidal; (2) the nature of the constituents of "liver-of-sulphur" which are of fungicidal value.

With regard to the strength at which a solution of "liver-of-sulphur" is fungicidal we find a diversity of statement. English writers[3] give the proportion of 1 oz. to 2-3 gallons (English) of water; i.e. a 0·31 % to 0·21 % solution; Sorauer[4] states that authors recommend a solution containing from 0·25 % to 0·4 %. It may be mentioned that it is not an uncommon practice among hop growers in this country when wishing to combat hop-mildew to add from 1 to 1½ lbs. of "liver-of-sulphur" to the 100 gallons of "hop-wash," i.e. to use a 0·1 % to 0·15 % solution of "liver-of-sulphur." American writers[5] recommend for use against mildews a solution containing 1 oz. to 2-4 gallons (American) of water, i.e. from 0·37 % to 0·18 %[6]. Lodeman[7] states that solutions containing from 0·19 % to 0·75 % of "liver-of-sulphur" are used, without stating what concentrations are used against specific diseases. Bourcart[8] mentions that Vesque recommends spraying with a 1 % solution of "liver-of-sulphur" against *Sphaerotheca pannosa*, although it is not stated whether this solution is intended for use on foliage or not. Hollrung[1] states that Mohr employed against *S. pannosa* on the Rose and Peach a 1·3 % solution of "liver-of-sulphur" containing 1·3 % glycerine, without stating whether the mixture was used in summer or winter. Against *S. mors-uvae*, the American Gooseberry-mildew, Goff[2] recommends the use of a 0·18 % to 0·37 % solution, and Close[3], Beach[4], and Duggar[5] a solution containing 0·37 %. Against the Vine-mildew (*Uncinula necator*) Galloway[6] recommends the 0·37 % solution; against the Cucumber-mildew (*Erysiphe Cichoracearum*) Humphrey[7] recommends as successful a 0·18 % solution.

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[1] *Idem, loc. cit.* p. 410.

[2] Chittenden, F. J., in *Journ. R. Hort. Soc.* XXXIX. 373 (1914).

[3] Massee, G., *Diseases of Cultivated Plants and Trees*, p. 56 (1910); Strawson, G. F., *Standard Fungicides*, p. 35 (1903).

[4] Sorauer, P., *Handbuch d. Pflanzenkrankheiten*, II. p. 525 (1908).

[5] Duggar, B. M., *Fungous Diseases of Plants*, p. 90 (1909); Stevens, F. L. and Hall, J. G., *Diseases of Economic Plants*, p. 34 (1910).

[6] It is probable that English copyists have repeated the American formulae, oblivious of the fact that the American gallon of water weighs only 8·34 lbs., and is therefore smaller than the English gallon which weighs 10 lbs.

[7] Lodeman, E. G., *The Spraying of Plants*, p. 163 (1903).

[8] Bourcart, E., *Insecticides, Fungicides, and Weedkillers*, p. 115 (1913) (English translation).

It has to be remembered that the substance "liver-of-sulphur" is not a chemical individual substance but rather a mixture of a great variety of sulphur compounds, chiefly sulphides and polysulphides of potassium (or sodium) and that its composition varies according to the mode of its preparation. Also, that its composition changes on the material being kept unless precautions are taken to avoid contact with the air. It is clear, therefore[8], that the composition of one sample of "liver-of-sulphur" may differ very widely from another sample which appears to the eye to be equally good, a fact which may perhaps explain to some extent the diversity found in the concentrations recommended for use.

The opinion which has been generally held regarding the mode of action of "liver-of-sulphur" appears to be that this substance is valuable by reason of the fact that on exposure to air its solutions readily deposit sulphur in an extremely fine state of division. It is believed in fact that the soluble constituents of the "liver-of-sulphur"—which are chiefly sulphides and polysulphides—do not act *per se*, although their presence is necessary as giving the required deposit of sulphur. Other views have been put forward attributing the fungicidal action to some oxidation product of the higher sulphides, e.g. the thiosulphate—either present in the spray fluid or produced after spraying on the plant.

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[1] Hollrung, M., *Handbuch d. chemischen Mittel gegen Pflanzenkrankh.* p. 44 (1898).

[2] Goff, E. S., in *Journ. of Mycology*, v. p. 33 (1889).

[3] Close, C. P., in *New York Agric. Exper. Stat.*, *Bull.* 161, p. 153 (1899).

[4] Beach, S. A., in *New York Agric. Exper. Station Bull.* p. 114 (1897).

[5] Duggar, B. M., *Fungous Diseases of Plants*, p. 223 (1909).

[6] Galloway, B. T., in *Journ. of Mycology*, VI. p. 13 (1891).

[7] Humphrey, J. E., in *Rep. Mass. State Agric. Exper. Stat.* IX 222 (1892), and X. 225 (1893).

[8] *Vide Journ. of Board Agric.* XXI. p. 236 (1914).

It is believed also that the alkaline nature of the solutions may aid the fungicidal action of the sulphur, although the sulphur which is deposited on the decomposition of the sulphides is believed to be the chief fungicidal agent. In a recent publication on this subject, Foreman[1] goes further than this as the result of a number of experiments with germinating spores of *Botrytis cinerea* and *Sphaerotheca mors-uvae*, and claims that the most potent fungicidal agent is the free alkali. From this point of view it is interesting to find that caustic alkalies and alkaline solutions have sometimes been used as fungicides. For example, solutions of sodium carbonate ranging from 0·05 % to 2·5 % have been found to inhibit the germination of the spores of certain fungi; a 1 % solution of ammonium carbonate perceptibly hinders the germination of *uredospores*[2].

It would obviously be unsafe, however, to assume that the concentration at which a fungicide is able to inhibit the germination of the spore is that at which it is fungicidal to the well-established actively-growing fungus. It has been pointed out by Wallace, Blodgett and Hesler[3] that a solution which gives satisfactory results when used against spores in the laboratory requires a concentration several hundred times stronger to control the same fungus when growing on the plant. Foreman[4] records that a 0·16 % solution of caustic soda prevents the germination of the spores of *Botrytis cinerea* and *Sphaerotheca mors-uvae*; we have found, however, that a 0·3 % solution does not kill well-developed patches of *S. Humuli*. It is clear that experiments based on the behaviour of spores placed in the fungicide have little practical value as indicating the strength at which the same substance will be fungicidal when used against the growing fungus on the plant.

A substance of more recent introduction for use against "powdery mildews" is iron sulphide. P. J. O'Gara speaks[5] of a spray-fluid containing 0·38 % iron sulphide as being "the standard summer-spray for apple and rose mildew" in fruit-growing districts in Oregon, U.S.A. In some field experiments carried out by one of us[6] in 1911 iron sulphide proved a powerful fungicide against the Apple "scab" fungus (*Fusicladium*). An "iron sulphide spray[1]" has been used by W. H. Volck[2] with success in field experiments against the Apple powdery mildew. M. B. Waite reports[3] the successful use of an "iron sulphide mixture" (to which arsenate of lead was added) against fungous diseases of the Apple. The striking results obtained in our experiments with a mixture of iron sulphide and soft soap are recorded below at p. 501.

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[1] Foreman, F. W., "The Fungicidal Properties of Liver of Sulphur" (*Journ. Agric. Science*, III. 401 (1910)).

[2] Hollrung, M., *Handb. d. chem. Mitt* pp. 49, 50.

[3] Wallace, E., Blodgett, F. M., and Hesler, L. R., "Studies of the Fungicidal Value of Lime-Sulfur Preparations" (*Cornell Univ. Agric. Exper. Station, Bull.* 290 (1911)).

[4] Foreman, F. W., *loc. cit.*

[5] *Leaflet*, Rogue River Valley, Medford, Oregon (1911).

[6] Salmon, E. S., "Report on Economic Mycology" (*Journ. S.-E. Agric. College*, XXI. p. 346 (1912)).

METHODS.

The plants used in testing the fungicidal value of the various solutions were 1- or 2-year old seedlings of the Hop (*Humulus Lupulus* Linn.) bearing the "powdery mildew" *Sphaerotheca Humuli* (DC.) Burr. The plants stood in an unheated greenhouse, kept as well ventilated as possible. In a few experiments in 1915 Gooseberry bushes in the open, bearing the American Gooseberry-mildew (*S. mors-uvae* (Schwein.) Berk.), were used.

The hop-plants used in the experiments were kept close together, and under the conditions of culture became severely infected with the hop-mildew. The spraying was done during the months of May, June and July. The plant used for spraying was carefully selected as bearing on a number of its leaves young and vigorously-growing patches of the mildew in its conidial stage. In order to make the experiments as strictly comparable as possible only those patches of mildew were used where the growth was so vigorous that the abundant conidiophores had produced masses of ripe, free conidia[4]. On each plant from 2 to 4 leaves, each bearing a large number (10–20) of "powdery" patches, were sprayed (using a hand "atomiser") with the solution, while the same number of leaves bearing exactly similar patches were reserved as "controls." The solution was applied in the finest spray with sufficient force and quantity to wet *thoroughly* all the patches of mildew. The "control" leaf was always on the same plant, and was usually the opposite leaf at the same node. In every experiment made, the mildew on the control leaves continued to grow and extend its patches; it is therefore unnecessary to mention the

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[1] The spray used is described by the author as being "a mixture of iron sulphide, gypsum and precipitated sulphur." Arsenate of lead was added to it. The percentage of iron sulphide in the various spray-fluids used was, apparently, from 0·2 % to 0·6 %.

[2] Volck, W. H., in *Better Fruit*, p. 39 (1911).

[3] Waite, M. B., *U.S. Dept. Agric. Bureau of Plant Industry Circular*, 58 (1910).

[4] This stage is denoted by the term "powdery" in the details of the experiments given below at p. 480 and sqq.

"controls" in the details of the experiments recorded below. The sprayed leaves were regularly observed at intervals of a few days from the first day after spraying until the action of the solution was determined. Owing to the superficiality of the mildew, and the ease with which the plants could be handled, no difficulty was experienced in accurately determining the biological condition of the fungus.

In preliminary trials it was found that when patches of mildew in an actively growing "powdery" condition are sprayed with aqueous solutions, the spray (even when very finely divided and applied with force) collects in minute drops on the surface of the densely packed conidiophores and conidia, being prevented by the presence of air at these places from uniformly wetting the fungus[1]. When, however, a 0·5 % or 1 % solution of soft soap is added, the wetting power of the solution used is increased to such an extent that it spreads evenly and uniformly through all "powdery" patches[2]. In the case of lime-sulphur, to which soap cannot be added for chemical reasons, 0·125 % or 0·25 % of saponin[3] was added, so as to secure, as far as possible, uniformity in the spreading properties of the sprays used.

MATERIALS USED.

For the sake of clearness, when considering the effect produced by the various spraying fluids used, a brief description of the materials comprising them is desirable. It should be mentioned in the first place that for the purpose of decreasing the surface tension of the spray-fluids and thus increasing their power of *wetting* powdery surfaces, definite quantities of soap have been used in the preparation of most of the solutions employed. In other cases, where for chemical reasons soap could not be used, as in the case of lime-sulphur, its place in the mixture has been taken by saponin[4].

Soap. In all cases where soap has been used alone or in conjunction with other substances the soap used was that known commercially as "Cook's Soap." The sample was rather a fluid type of soft soap which exhibited a neutral or slightly acid character. The total alkali was found to be equal to 11·6 % KOH (or 8·36 % NaOH).

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[1] Mr S. U. Pickering has already called attention to this fact (*11th Report Woburn Exper. Fruit Farm*, p. 119 (1910)).

[2] In some spraying experiments with certain washes carried out under practical conditions in the open, Messrs Barker and Lees (*Report Agric. and Hort. Research Station, Long Ashton*, for 1914, p. 73) found that 2 °/₀ soft soap solution did not thoroughly wet the mildew, while a 2 °/₀ paraffin emulsion did so.

[3] Mr Pickering (*loc. cit.* p. 159) has pointed out that the action of saponin resembles that of soap in increasing the wetting properties of spray-fluids.

[4] *Vide 11th Report Woburn Exper. Fruit Farm*, p. 159 (1910).

Saponin. The material used was the ordinary white powder sold commercially.

Liver-of-sulphur. Of several samples examined, one supplied by Messrs Baird and Tatlock was selected as being a good sample and suitable for the purpose of this work. It was found to contain 44 % sulphur of which 42·2 % was present as sulphide-sulphur[1]; less than 1 % as sulphate and less than 1 % as sulphite and thiosulphate. The alkalinity was found to be equal to 4–5 % K_2CO_3 and the total alkali, calculated as KOH, equal to 59·6 % of which 48·8 % was due to potassium. The sample may be considered to be a superior one to anything likely to be purchased by the grower.

Yellow ammonium sulphide. This material was prepared by saturating 200 c.c. of a 10 % solution of ammonia in water at 17° C. with sulphuretted hydrogen, then adding 400 c.c. of 10 % ammonia solution and 1000 c.c. of water. To this mixture 21 grms. of flowers of sulphur were added and when completely dissolved the clear solution constituted the stock solution used throughout this work[2]. The total sulphur present in this solution was found to be 3·7 % of which 2·2 % was present as sulphide-sulphur. Sulphates were absent and only traces of sulphites and thiosulphates could be detected. The sp. gr. of the stock solution was 1·001 at 15° C.

Colourless ammonium hydrosulphide. This was prepared by saturating a 4 % solution of ammonia in water with sulphuretted hydrogen. The amount of "sulphide-sulphur" which was present was found to be 6·72 %.

Colourless ammonium sulphide. The above solution of ammonium hydrosulphide was mixed with an equal volume of 4 % ammonia solution. The quantity of sulphide-sulphur was found to be 3·36 %.

Lime-sulphur. Berger's brand of "lime-sulphur" was used and diluted to the sp. gr. 1·01 and 1·005. At the former concentration the amount of sulphide-sulphur present was found to be 1·43 %.

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[1] The estimation of sulphide-sulphur was effected by the volumetric method in which a standard ammoniacal solution of zinc is employed and a solution of nickel sulphate used as an outside indicator.

[2] The method followed in preparing the spray-fluids from the stock solution may be illustrated in the case of that used in Expers. 9 and 10, p. 494, which was prepared by diluting 25 c.c. of stock solution to 200 c.c. with distilled water and then adding 200 c.c. of a 2 % solution of soft soap in distilled water.

Sodium thiosulphate. The ordinary commercial crystalline material was used.

Ammonia. The ammonia solutions used were prepared from a strong solution which contained 24·7 % ammonia (sp. gr. 0·911 at 15° C.) by diluting it with water.

Caustic soda. The substance used was that usually sold in stick form.

Hydrogen sulphide. Distilled water, after having been boiled to expel air, was cooled and saturated with the purified gas. The amount of sulphide-sulphur present was found to be 0·113 %.

Iron sulphide. Two methods were followed in the preparation of this material: (i) a weighed quantity of crystallised ferrous sulphate was dissolved in water and a dilute solution of yellow ammonium sulphide added drop by drop until the formation of iron sulphide seemed complete; (ii) a dilute solution of ferrous sulphate was added to a known quantity of ammonium hydrosulphide solution until no further precipitation occurred. It should be mentioned that when made according to method (i) the iron sulphide remains longer in a fine state of division and is consequently in a better form for applying as a fine spray.

I. VARIOUS SUBSTANCES.

Soft soap.

Hops. 1914.

Exper. 1. *Solution containing* 1 % *soft soap.*

2nd day. The majority of the patches showing a vigorous growth of conidiophores.

3rd day. All the patches of mildew now as vigorous and as "powdery" as before spraying. There was no injury to the leaf-cells at any place.

1915.

Exper. 1. *Solution containing* 1 % *soft soap.*

2nd day. The patches of mildew scarcely checked; all showing an abundant growth of young conidiophores.

3rd day. All the patches now "powdery."

Saponin.

Hops. 1914.

Exper. 1. *Solution containing* 0·25 % *saponin.*

1st day. Mildew little affected.

3rd day. All the patches of mildew vigorous and "powdery."

Sodium carbonate.

Hops. 1915.

Exper. 1. *Solution containing* 0·3 % *carbonate of soda* (*crystallised*) *and* 1 % *soft soap.*

2nd day. The mildew scarcely checked; all the patches with numerous immature conidiophores.

3rd day. All the patches now densely powdery.

Sodium thiosulphate.

Hops. 1915.

Exper. 1. *Solution containing* 1 % *sodium thiosulphate and* 1 % *soft soap.*

2nd day. The mildew but little checked; some patches almost "powdery."

8th day. All the patches as "powdery" as on the controls. No injury to the leaf.

Summary of Observations. I.

None of the following substances—soft soap[1], 1 % solution; saponin, 0·25 %; sodium carbonate[2], 0·3 % and soft soap, 1 %; sodium thiosulphate[3], 1 % and soft soap, 1 %- had any fungicidal value.

II. CAUSTIC SODA.

Hops. 1915.

Exper. 1. *Solution containing* 0·3 % *caustic soda and* 1 % *soft soap.*

2nd day. All the patches much checked, with conidiophores all collapsed. Most of the patches with dark rims round them; otherwise no injury to the leaves.

3rd day. All the patches greatly checked, mostly dormant and sterile; a few with, here and there, isolated conidiophores bearing chains of spores.

6th day. Some of the patches with fairly numerous small groups of conidiophores near the centre; most with only a very few scattered conidiophores; some of the patches dead.

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[1] See above, p. 478.

[2] G. Dorogin has stated (*Zeitschr. f. Pflanzenkrankh.* XXIII. p. 335 (1913)) that a solution of 0·25 % or 0·5 % of carbonate of soda or carbonate of potash is efficacious against the American Gooseberry-mildew. Hector, J. M. and Auld, S. J. M. (*Gardeners' Chronicle*, Aug. 7, 1915, pp. 79–80) believe that they obtained some evidence in field experiments that a 0·3 % solution of carbonate of soda was detrimental to the American Gooseberry mildew.

[3] Hollrung, *loc. cit.* p. 50, mentions that Hitchcock and Carleton (*Kansas Exper. Station, Bull.* 38) state that the germinating capacity of uredospores is weakened by prolonged treatment with a 1 % solution of sodium thiosulphate.

8th day. The mildew alive on the majority of the patches and forming small groups of conidiophores or scattered single conidiophores. The leaf-cells at the periphery of each patch brown and dead; microscopical examination showed that the epidermal cells alone at these places were killed.

Exper. 2. *Solution containing* 0·5 % *caustic soda and* 1 % *soft soap.*

1st day. The patches all much checked, with the epidermal leaf-cells at the periphery of each patch darkened. The tip of one leaf, where the fluid had collected, shrivelled.

3rd day. The mildew greatly checked; some patches bearing a few isolated conidiophores towards the centre.

7th day. Most of the patches now showing a vigorous growth of conidiophores—some patches almost powdery.

13th day. Most of the patches quite powdery.

Exper. 3. *Solution containing* 0·5 % *caustic soda and* 1 % *soft soap.*

3rd day. Mildew checked; some patches showing a few conidiophores. The older leaves slightly "scorched" at the tip; the youngest leaves severely "scorched" at the edge and tip; growing tip of shoot not injured.

6th day. Mildew greatly checked; most of the patches dead; the others showing short, weak conidiophores. No further injury produced.

9th day. The patches of mildew now showing vigorous, erect conidiophores; some of the patches "powdery."

Exper. 4. *Solution containing* 0·75 % *caustic soda and* 1 % *soft soap.*

3rd day. Mildew dead or very greatly checked. All the leaves severely "scorched," the injury being in the form of dead patches of cells chiefly at the tip and edges of the leaf, but occasionally elsewhere; the growing tip of shoot not injured. (The "scorching" of the youngest leaves was sufficiently serious to prevent them subsequently from developing normally.)

6th day. All the patches nearly but not quite dead—only a few weak conidiophores present.

9th day. All the patches dead.

Exper. 5. *Solution containing* 1 % *caustic soda and* 1 % *soft soap.*

1st day. The mildew greatly checked; the epidermal cells at the periphery of each patch darkened. The tip of one leaf slightly shrivelled.

3rd day. Most of the patches dormant, and some dying or dead; a few with bases of young conidiophores. No further injury, which was very slight.

7th day. No further growth of the mildew.

9th day. A few of the patches with small clusters of conidiophores.

13th day. A few patches showing small but vigorous tufts of conidiophores; the majority dead or nearly so.

Exper. 6. *Solution containing* 1 % *caustic soda and* 1 % *soft soap.*

1st day. Mildew greatly checked; the leaf-cells underlying the patches turned brownish-black, the injury extending in most cases to the under-surface.

4th day. All the patches dead or dying.

6th day. A few patches showing a few scattered conidiophores at the centre.

10th day. The mildew now practically everywhere dead; no further injury to the leaves.

Exper. 7. *Solution containing* 1 % *caustic soda and* 1 % *soft soap.*

1st day. All the patches of mildew killed, accompanied by the death of the underlying leaf-cells. The tip of each leaf blackened and shrivelled.

4th day. Distinct injury apparent to the tip of each leaf and the margins near the tip.

Exper. 8. *Solution containing* 1·5 % *caustic soda and* 1 % *soft soap.*

1st day. All the patches of mildew greatly checked or killed; accompanied by a blackish-brown discoloration of the underlying leaf-cells, the injury extending to the lower surface.

4th day. On the upper leaves all the patches dead; on the lower leaves most of the patches dead, but some bearing a very few scattered conidiophores at the centre. No further injury to the leaves.

6th day. Only one patch on a lower leaf alive and showing a very few scattered conidiophores.

10th day. The one patch of mildew nearly dead.

Exper. 9. *Solution containing* 1·5 % *caustic soda and* 1 % *soft soap.*

1st day. The mildew greatly checked, or killed; slight injury to the tip and edge of each leaf.

2nd day. All the patches apparently killed; decided injury to the oldest leaves in the form of pale "burnt" areas and curling of margins; to the next oldest leaves in the form of curling of the tip and margins; to the youngest leaves in slight injury to the tip.

6th day. The mildew entirely killed; no further injury to the leaves (this was, however, serious).

Exper. 10. *Solution containing* 1·5 % *caustic soda and* 1 % *soft soap.*

3rd day. The mildew greatly checked. All the leaves badly "scorched,"—the two oldest leaves severely "scorched" at their margins; the two intermediate leaves less badly "scorched"; the youngest leaves so badly "scorched" at their margins that they never developed normally.

9th day. All the patches of mildew dead.

Exper. 11. *Solution containing* 2 % *caustic soda and* 1 % *soft soap.*

1st day. The mildew very greatly checked. All the leaves severely "scorched," the tips and margins curled.

2nd day. All the patches of mildew killed, accompanied by the death of the underlying leaf-cells. All the leaves seriously injured, curled and shrunken at the margins and showing pale "burnt" areas over their surface.

Gooseberries. 1915.

For the purpose of ascertaining the effect of a caustic soda spray upon Gooseberry foliage, a solution of 1·5 % was used on the leaves of two varieties of Gooseberry, viz. Yellow Rough and Lancashire Lad. On Yellow Rough it produced by the first day "scorching" in the form of minute black patches on the youngest leaves only; the extreme tip of one shoot was killed. On the fourth day some of the older leaves showed patches of a brown discoloration and also brown edges; by the tenth day a severe defoliation had occurred. On Lancashire Lad by the first day the youngest leaves only showed "scorching" in the form of minute black patches; on the fourth day some of the older leaves showed brown edges, and by the tenth day a few of the leaves had fallen.

Summary of Observations. II.

Caustic soda was not tried at a lower concentration than 0·3 %. At this strength it has an immediate injurious action on the mildew and at the same time kills the epidermal cells at the periphery of the mycelium of the mildewed patch. The action is not powerful enough, however, at this concentration, nor at the increased concentration of 0·5 %, to kill the mildew, and we find that the mildew recovers gradually until by the ninth to thirteenth day after treatment many of the patches are again powdery with conidia from fresh conidiophores.

Serious injury to the leaf (apart from the portion occupied by the mildew) has been observed at a concentration of 0·75 % which almost prohibits the use of such a solution. At 1 % the mildew is usually killed and the tip of the leaf is usually "scorched" and killed. In Exper. 7 (recorded above), in which the three mildewed leaves were sprayed on both sides with a 1 % solution, all the patches of mildew were killed very satisfactorily and this was accompanied by the death of the leaf-cells underlying and surrounding each patch. Distinct "scorching" injury was produced to the tip of the leaf and adjacent margin.

At the concentration of 1·5 % the risk of serious leaf-injury is so great as to prohibit the use of such a solution. In Exper. 10, the older leaves were very seriously scorched by a 1·5 % solution and while the younger leaves showed only injury at the tip, it was found that this so damaged them that they could not develop properly. In one experiment (not recorded above) twelve hop plants, bearing numerous patches of mildew on many of the leaves, were thoroughly sprayed with a 1·5 % solution. In this experiment the whole plants were treated, including the youngest leaves and the growing tip of the stem, and each leaf was sprayed on both surfaces. The injury produced by the third day was very marked: many of the leaves were completely discoloured and were more or less rigid, and dying or dead—at a touch they fell to the ground—whereas others were only injured severely at the tip and edges. The growing tips of two plants were killed.

Taking into consideration also the effect produced by this material on gooseberry foliage it seems clear that caustic soda by itself (or with soap) is unlikely to prove a satisfactory fungicide, although the fact that a 0·3 % solution exerts a partial fungicidal action must be taken into account when considering the value of other substances exhibiting alkaline properties.

III. AMMONIA.

Hops. 1915.

Exper. 1. *Solution containing* 0·5 % *ammonia and* 1 % *soft soap.*

1st day. All the patches white and apparently only slightly checked; the bases of the conidiophores everywhere visible.

4th day. The patches powdery or nearly so.

6th day. All the patches powdery.

Exper. 2. *Solution containing* 1 % *ammonia and* 1 % *soft soap.*

1st day. The patches white, slightly checked; bases of conidiophores everywhere visible.

4th day. All the patches powdery or nearly so.

6th day. All the patches as powdery as those on the "control" leaves.

Exper. 3. *Solution containing* 1·5 % *ammonia and* 1 % *soft soap.*

2nd day. The mildew little checked—some of the patches almost powdery. Slight injury to the leaf in the form of minute, brown, "burnt" patches of cells—one or two on each of the three sprayed leaves.

8th day. All the patches now densely powdery; no further injury to the leaf.

Exper. 4. *Solution containing* 2 % *ammonia and* 1 % *soft soap.*

2nd day. The mildew little checked, some of the patches almost powdery. Slight injury to the leaf in the form of minute, brown, "burnt" patches of cells—two or three on each of the three sprayed leaves; the tip of one leaf injured also.

8th day. All the patches now densely powdery; no further injury to the leaf.

Exper. 5. *Solution containing* 4 % *ammonia and* 1 % *soft soap.*

1st day. The mildew much checked on lowest leaf; on the uppermost leaf several of the patches bore short conidiophores. Injury to the lower leaves in the form of slight discoloration (darkening) and curling of the margin.

3rd day. The mildew much checked; most of the patches on the lowest and middle leaves apparently dead; on the uppermost leaf several of the patches with small groups of conidiophores. Injury more apparent; the lowest leaf killed and curled at the margins; the middle leaf with injury in the form of small, brown, "burnt" patches of cells; the uppermost leaf not injured.

7th day. All the leaves now with several patches of mildew bearing vigorous tufts of conidiophores.

Exper. 6. *Solution containing* 8 % *ammonia and* 1 % *soft soap.*

Within two hours of spraying the sprayed leaves had curled, withered, and were dead or dying.

1st day. All the leaves dead and shrivelled.

Summary of Observations. III.

Ammonia at 4 % severely checks the mildew, but does not kill all the patches; at this strength serious injury is produced to the leaf-tissue. At 2 % slight injury to the leaf begins to be caused, in the form of small "burnt" areas scattered over the leaf without reference to the disposition of the patches of mildew; at this concentration ammonia is practically without fungicidal value, some of the patches of mildew becoming almost "powdery" the second day after treatment.

IV. "LIVER-OF-SULPHUR[1]."

Hops. 1914.

Exper. 1. *Solution containing* 0·3 % *liver-of-sulphur* (0·13 % *sulphide sulphur* (S.S.)) *and* 1 % *soft soap.*

1st day. Mildew checked.

3rd day. Mildew still checked, but a few fresh conidiophores beginning to be developed on some of the patches.

5th day. Many of the patches now with groups of conidiophores.

8th day. Most of the patches now more or less "powdery."

Exper. 2. *Solution containing* 0·3 % *liver-of-sulphur* (0·13 % S.S.) *and* 0·25 % *saponin.*

1st day. Mildew checked.

3rd day. All the patches beginning to produce conidiophores.

6th day. All the patches now "powdery."

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[1] *Vide* Materials used, p. 478.

Exper. 3. Four leaves (each covered with numerous "powdery" patches of mildew) at two nodes were sprayed with the two following solutions, (*a*) and (*b*)—one leaf at each node being used for each solution.

(*a*) *Solution containing* 0·3 % *liver-of-sulphur* (0·13 % S.S.) *and* 1 % *soft soap.*

(*b*) *Solution of yellow ammonium sulphide containing* 0·16 % S.S. *and* 1 % *soft soap.*

1st day. (*a*) and (*b*) Mildew checked.

2nd day. (*a*) All the patches showing renewed growth and developing very numerous conidiophores.

(*b*) All the patches dormant and sterile.

5th day. (*a*) Most of the patches now "powdery"; the remaining patches "subpowdery."

(*b*) As on the 2nd day.

7th day. (*a*) and (*b*) As on the 5th day

11th day. (*a*) All the patches of mildew as vigorous and as "powdery" as though they had never been sprayed.

(*b*) All the patches now dead or dying.

Exper. 4. Opposite leaves bearing numerous "powdery" patches of mildew were sprayed with

(*a*) *Solution containing* 0·3 % *liver-of-sulphur* (0·13 % S.S.) *and* 1 % *soft soap.*

(*b*) *Solution of yellow ammonium sulphide containing* 0·08 % S.S. *and* 1 % *soft soap.*

It was noticeable that the spray did not permeate so well in (*a*) as in (*b*). When the spray was dry, the mildew patches on the leaf sprayed with (*a*) were white in colour, and showed numerous erect conidiophores; with (*b*) the patches were a dingier white, and nearly all the conidiophores had collapsed.

1st day. (*a*) Mildew apparently vigorous and growing.

(*b*) Mildew apparently dormant.

3rd day. (*a*) and (*b*) As above.

5th day. (*a*) Mildew now showing erect clustered conidiophores.

(*b*) Mildew still dormant and sterile.

9th day. (*a*) The patches of mildew now "powdery."

(*b*) The patches of mildew dormant and sterile.

12th day. (*a*) and (*b*) As above.

Exper. 5. *Solution containing* 0·4 % *liver-of-sulphur* (0·17 % S.S.).

1st day. Mildew slightly checked.

4th day. Many patches now with vigorous erect conidiophores.

6th day. Most of the patches "powdery" or "subpowdery."

Exper. 6. *Solution containing* 0·4 % *liver-of-sulphur* (0·17 % S.S.) *and* 1 % *soft soap.*

1st day. Mildew checked.

3rd day. Mildew still much checked.

5th day. Many patches still much checked, and sterile; a few patches now showing scattered conidiophores, or scattered groups of same.

8th day. Most of the patches with groups of vigorous clustered conidiophores.

Exper. 7. *Solution containing* 0·4 % *liver-of-sulphur* (0·17 % S.S.) *and* 1 % *soft soap.*

1st day. Mildew checked.

4th day. All the patches still checked and sterile.

6th day. As above.

10th day. Most of the patches still quite sterile; two or three patches only producing a few tiny clusters of new conidiophores.

Exper. 8. *Solution containing* 0·4 % *liver-of-sulphur* (0·17 % S.S.) *and* 0·25 % *saponin.*

1st day. Mildew checked.

3rd day. On one leaf (*a*) all the patches dormant and sterile; on the other leaf (*b*) some patches producing a few conidiophores.

5th day. (*a*) as above; (*b*) the patches growing vigorously and almost "powdery."

8th day. (*a*) as above; (*b*) most of the patches now "powdery."

Exper. 9. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.).

1st day. Mildew slightly checked.

4th day. Some of the patches killed and brown, with death of the subjacent cells extending through to the under-surface of the leaf; other patches living and showing a few erect conidiophores.

6th day. As above.

10th day. The majority of the patches dead; a few patches bearing a few scattered conidiophores.

Exper. 10. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.) *and* 1 % *soft soap.*

1st day. Mildew checked.

5th day. All the patches checked and sterile.

8th day. Many of the patches quite sterile; a very few patches with a few conidiophores, but none vigorous.

Exper. 11. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.) *and* 1 % *soft soap.*

1st day. Patches all checked, dormant and sterile.

4th day. One leaf with all the patches dingy white, sterile and apparently dying; the other leaf with most of the patches sterile, but with one or two patches showing fresh growth.

10th day. On one leaf all the patches dead; on the other leaf all the patches dead except two, where tiny clusters of new conidiophores were visible.

Exper. 12. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.) *and* 1 % *soft soap.*

1st day. Mildew checked, but many conidiophores still visible.

3rd day. The conidiophores still visible on many patches, but no further development of others.

8th day. All the patches greatly checked, many quite sterile and dead or dying; on each leaf, however, two or three patches showed minute groups of conidiophores, usually towards the centre of the patch.

12th day. Each leaf now with some patches bearing vigorous conidiophores—here and there even "powdery."

Exper. 13. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.) *and* 0·5 % *soft soap.*

1st day. Mildew checked, but erect conidiophores visible.

3rd day. All the patches still greatly checked.

5th day. Many patches on both leaves developing conidiophores; a few patches almost "powdery."

8th day. As above.

12th day. Many patches now almost or quite "powdery."

Exper. 14. *Solution containing* 0·6 % *liver-of-sulphur* (0·26 % S.S.) *and* 0·25 % *saponin.*

1st day. Mildew checked.

3rd day. As above.

5th day. Most of the patches still checked: a few patches producing a few conidiophores.

8th day. All the patches now producing abundant conidiophores.

Exper. 15. *Solution containing* 0·8 % *liver-of-sulphur* (0·34 % S.S.).

1st day. Patches of mildew more or less checked.

4th day. Most of the patches showing some erect conidiophores.

6th day. Mildew very severely checked; some of the patches brown and dead, accompanied by the death of the whole area of the subjacent leaf-cells (extending through to the under-surface of the leaf); some patches showing a few conidiophores.

10th day. Nearly all the patches killed; a few still bearing a few scattered conidiophores.

Exper. 16. *Solution containing* 0·8 % *liver-of-sulphur* (0·34 % S.S.) *and* 1 % *soft soap.*

1st day. All the patches of mildew checked, dormant and sterile.

4th day. All the patches of mildew quite sterile (the tips of both of the leaves yellow and shrivelled, due to the accumulation there of the fluid).

6th day. All the patches sterile; no further injury to the leaves.

10th day. All the patches dead.

Gooseberries. 1915.

In one experiment two shoots of a Gooseberry bush in the open infested with the American Gooseberry-mildew (*Sphaerotheca mors-uvae*) in its "powdery" conidial stage were sprayed in July, with respectively a solution of "liver-of-sulphur" containing 0·13 % sulphide-sulphur and a solution of ammonium sulphide containing 0·13 % sulphide-sulphur, both solutions containing 1 % soft soap. On the 7th day after spraying the shoots were examined; the "liver-of-sulphur" solution had had no more effect on the mildew than water, the mildew being now densely "powdery" with fresh conidiophores and conidia; the mildew on the shoot sprayed with ammonium sulphide was entirely sterile and obviously dying. No injury was caused to the foliage.

Summary of Observations. IV.

The experiments afford clear evidence that a 0·3 % solution of "liver-of-sulphur"—which, as already observed[1], is the strength which in this country has been generally recommended for use—is quite inefficacious as a fungicide against Hop and Gooseberry mildews. The patches are checked, more or less, for the first few days, but by about the 3rd day a fresh growth of conidiophores has taken place and, by the 5th to 8th day after spraying, the patches have become "powdery" again. The worthlessness of the 0·3 % solution of "liver-of-sulphur" (containing 0·13 % sulphide-sulphur) as compared with solutions of ammonium sulphide containing either 0·08 % or 0·16 % sulphide-sulphur was shown clearly in those experiments with Hops (Nos. 3 and 4) where mildewed leaves on the same plant were sprayed with the two solutions and also in the case of the Gooseberries sprayed in the open.

A solution containing 0·4 % of "liver-of-sulphur" (0·17 % sulphide-sulphur) without the addition of soft soap has no greater fungicidal value than the 0·3 % solution; with the addition of soft soap the patches are checked, but they are not killed entirely and are able to produce conidiophores by the 8th to 10th day.

A solution containing 0·6 % "liver-of-sulphur" (0·26 % sulphide-sulphur) greatly checks the mildew, which may remain dormant permanently or may produce a few tiny clusters of conidiophores. In two experiments, however, where this solution was used with respectively 1 % and 0·5 % soft soap, the patches of mildew after being greatly checked recovered, produced fresh conidiophores by the 5th to 8th day and were almost or quite powdery by the 12th day.

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[1] See p 474.

When the concentration of "liver-of-sulphur" is increased to 0·8 % (0·34 % sulphide-sulphur) the solution proved either completely or almost completely fungicidal.

It must be observed, however, when considering the fungicidal action of the solutions referred to above that with Gooseberries serious scorching occurs[1] at concentrations greater than 0·3 %, at which concentration it is found that the fungicidal value of this substance is nil.

V. AMMONIUM SULPHIDE (YELLOW).

Hops. 1914.

Exper. 1. *Solution containing* 0·04 % *sulphide-sulphur* (S.S.) *and* 1 % *soft soap.*

1st day. Mildew much checked.
3rd day. All the patches still dormant and sterile.
5th day. As above.
7th day. All the patches quite sterile and many of them dying.
12th day. All the patches dying or dead.

Exper. 2. *Solution containing* 0·04 % S.S. *and* 0·5 % *soft soap.*

1st day. Mildew checked; short conidiophores visible on most patches.
3rd day. As above.
5th day. All the patches still dormant.
8th day. All the patches completely sterile and many dying or dead.
12th day. Mildew just alive at two or three patches only (out of 20 patches), and producing there a very few conidiophores; the remaining patches dead.

Exper. 3. *Solution containing* 0·06 % S.S. *and* 1 % *soft soap.*

1st day. Mildew checked.
3rd day. Patches either sterile or with very short conidiophores.
5th day. All the patches quite sterile, dormant or dying.
8th day. As above.

Exper. 4. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

1st day. All the patches checked and dormant.
3rd day. All the patches still quite dormant.
5th day. All the patches dormant and sterile.
7th day. All the patches sterile, some dying.
9th day. As above.

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[1] Salmon, E. S., "Report on Economic Mycology" (*Journ. S.-E. Agric. Coll.* XXII. p. 410 (1913) [1914]).

Expers. 5, 6, 7, 8. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

The results obtained in all these experiments were the same as that recorded above in Exper. 4, all the patches of mildew being sterile, and either dormant, dying or dead by the 9th to 12th day.

Exper. 9. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

In this experiment the action of the ammonium sulphide was compared with that of a solution of "liver-of-sulphur" containing 0·13 % sulphide-sulphur. The results are given under "*Liver-of-sulphur,*" Exper. 4 (see p. 487).

Exper. 10. *Solution containing* 0·16 % S.S. *and* 1 % *soft soap.*

In this experiment the action of the ammonium sulphide was compared with that of a solution of "liver-of-sulphur" containing 0·13 % sulphide-sulphur. The results are given under "*Liver-of-sulphur,*" Exper. 3 (see p. 487).

1915.

Exper. 1. *Solution containing* 0·02 % S.S. *and* 1 % *soft soap.*

1st day. Mildew checked; patches showing bases of conidiophores in large numbers.

4th day. Conidiophores remaining short.

7th day. Conidiophores still remaining short, even where very numerous; a very few isolated groups of taller conidiophores on a few patches; some patches quite sterile. The mildew obviously checked everywhere, and nowhere with "powdery" patches.

10th day. A few patches with small groups of conidiophores.

13th day. The patches increasing in size.

Exper. 2. *Solution containing* 0·03 % S.S. *and* 1 % *soft soap.*

1st day. Mildew much checked; mycelium more or less collapsed and flocculent; very few, weak, isolated conidiophores visible.

4th day. The patches all much checked and mostly sterile—a few with weak conidiophores.

7th day. Most of the patches sterile with collapsed mycelium; some patches with weak short conidiophores; three patches showing very small groups of vigorous erect conidiophores.

10th day. Four patches showing a very few erect conidiophores, the rest sterile.

13th day. Several patches with small tufts of conidiophores.

Exper. 3. *Solution containing* 0·04 % S.S. *and* 1 % *soft soap.*

1st day. Mildew much checked; mycelium more or less collapsed and flocculent; very few weak, isolated conidiophores visible.

4th day. A few patches showing a few weak conidiophores; most of the patches sterile.

7th day. As above.

10th day. A very few small tufts of conidiophores on a few patches on both leaves.

13th day. A few, small, almost powdery patches on both leaves.

Exper. 4. *Solution containing* 0·06 % S.S. *and* 1 % *soft soap.*

1st day. Mildew much checked; mycelium more or less collapsed and flocculent; very few, weak, isolated conidiophores visible.

4th day. The mildew greatly checked, but not apparently dead.

7th day. On two leaves all the patches showed the mycelium collapsed and sterile or bearing only weak, short conidiophores; on the other two leaves some patches were as above described, while others showed little tufts of vigorous, longer conidiophores.

10th day. On one plant the two fairly old leaves bore nearly all sterile patches, a few only showing freshly-produced, erect conidiophores; on one plant the two leaves each bore the majority of patches with very numerous erect, almost or quite powdery conidiophores.

Exper. 5. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

1st day. Mildew much checked; mycelium more or less collapsed and flocculent; very few, weak, isolated conidiophores visible.

4th day. The patches white, but quite sterile.

7th day. The mycelium everywhere floccoso-collapsed, usually sterile but occasionally with minute conidiophores.

10th day. All the patches quite sterile or occasionally with very short and weak conidiophores.

13th day. As above.

18th day. All the patches, though still white, quite sterile.

Exper. 6. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

1st day. The mildew greatly checked, the mycelium all collapsed and flocculent.

3rd day. A very few conidiophores present on a few patches.

9th day. The patches mostly completely sterile; a few scattered conidiophores present on a few patches.

11th day. As above.

Exper. 7. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

1st day. The mycelium everywhere collapsed.

3rd day. All the patches quite sterile.

7th day. The patches all sterile, and dormant or dying.

10th day. As above.

Exper. 8. *Solution containing* 0·11 % S.S. *and* 1 % *soft soap.*

1st day. Mildew greatly checked, with mycelium collapsed.

3rd day. Some short conidiophores present on some patches.

7th day. Mycelium of all the patches collapsed, sterile and apparently dying.

10th day. The mycelium of many of the patches almost disappeared.

15th day. A few scattered conidiophores visible on a few patches.

Exper. 9. *Solution containing* 0·13 % S.S. *and* 1 % *soft soap.*

1st day. Mildew greatly checked, with mycelium collapsed.

3rd day. All the patches showing completely collapsed and flocculent mycelium, quite sterile.

7th day. All the patches apparently dying.

10th day. As above.

15th day. All the patches with quite sterile, collapsed mycelium and evidently dying.

Exper. 10. *Solution containing* 0·13 % S.S. *and* 1 % *soft soap.*

1st day. Mildew greatly checked, with mycelium collapsed.

8th day. All the patches with collapsed, sterile mycelium.

11th day. The mildew still white, but quite sterile and dying.

Exper. 11. *Solution containing* 0·16 % S.S. *and* 1 % *soft soap.*

1st day. Mildew greatly checked, with mycelium collapsed, short conidiophores visible on a few patches; no injury apparent to the leaves.

3rd day. The short conidiophores still visible.

7th day The patches of mildew dead (with collapsed and partially disintegrated mycelium) on the young leaves, which showed no injury from the spray; the two older sprayed leaves curled up and turning brown, while the control leaves remained uninjured.

10th day. The two young sprayed leaves now turning yellow and dying, and falling at a touch.

Exper. 12. *Solution containing* 0·16 % S.S. *and* 1 % *soft soap.*

1st day. Minute injury apparent at the tips of two of the leaves; patches showing short, old conidiophores.

3rd day. The old conidiophores still visible.

8th day. All the patches with collapsed, sterile mycelium; the young leaves all seriously injured at their tips.

11th day. The tips of all the sprayed leaves scorched and brown; large brown dead areas where the patches of mildew had been.

Gooseberries. 1915.

With the object of ascertaining the behaviour of the leaves to ammonium sulphide before using the solution against American Gooseberry-mildew the following three experiments were carried out. Gooseberry bushes in pots in a greenhouse were used and the leaves, fully-grown berries and tips of shoots were sprayed. Two varieties of Gooseberry were used.

Exper. 1. (*Lancashire Lad.*) *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

9th day. Very slight injury apparent in the form of the yellowing and dropping off of a few leaves.

11th day. No further injury. The whole of the injury caused was not appreciable from the practical (commercial) standpoint.

Exper. 2. (*Yellow Rough.*) *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

9th day. A large number of the leaves turned yellow, resulting in a severe leaf-fall.

11th day. A large number of the leaves, and also of the berries, had fallen off. The injury caused was so severe as to preclude the possibility of the use of the spray on this variety[1].

Exper. 3. (*Lancashire Lad.*) *Solution containing* 0·16 % S.S. *and* 1 % *soft soap.*

8th day. Decided scorching effect on the older leaves, accompanied by a slight leaf-fall. Two berries fell off.

11th day. No further injury caused.

In the following experiments Gooseberry bushes, growing in the open, affected with American Gooseberry-mildew (*Sphaerotheca mors-uvae*) in the "powdery" conidial stage, were sprayed.

Exper. 4. *Solution containing* 0·08 % S.S. *and* 1 % *soft soap.*

Six shoots, densely smothered with the mildew, were sprayed.

12th day. Four out of the six shoots bore only sterile mycelium; one shoot showed a few scattered conidiophores on the patches on two leaves; the remaining shoot had several minute, almost "powdery" patches on two leaves. It was clear that the solution had exerted a powerful fungicidal action.

Exper. 5. *Solution containing* 0·13 % S.S. *and* 1 % *soft soap.*

One shoot, densely smothered with the mildew in a "powdery" condition, was sprayed.

8th day. The mycelium everywhere dried up and completely barren. No injury caused to the leaves or tip of shoot. (NOTE. A similarly-affected shoot on the same bush was sprayed at the same time with a solution of "liver-of-sulphur" containing the same percentage (0·13 %) of sulphide-sulphur and 1 % soft soap. This had no fungicidal effect (see above, p. 490).)

Exper. 6. *Solution containing* 0·16 % S.S. *and* 1 % *soft soap.*

Six shoots, densely smothered with the mildew in a "powdery" condition, were sprayed.

12th day. The mildew on all six shoots was either dead, with the mycelium becoming disintegrated, or quite sterile and dying. No injury was caused to the leaves, berries or growing tips of the shoots, while the fungicidal action of the solution appeared to be complete.

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[1] This variety has proved to be extremely susceptible to injury from the effects of sulphur (see Salmon, "Report on Economic Mycology" (*Journ. S.-E. Agric. College*, XXII. p. 405 (1913) [1914]).

Summary of Observations. V.

In 1914 proof was obtained that a solution of ammonium sulphide containing 0·04 % sulphide-sulphur was powerful as a fungicide, while solutions containing 0·06 %, 0·08 % and 0·16 % sulphide-sulphur proved uniformly efficacious in rendering the mildew permanently sterile, and usually in reducing it by the 5th to 8th day to a dead or dying condition.

In 1915 solutions containing 0·02 %, 0·03 % and 0·04 % sulphide-sulphur were found to be too weak to be a perfectly satisfactory fungicide. When the concentration of sulphide-sulphur was 0·08 % the solution was fungicidal in some cases, in others it was not quite so; at 0·13 % the solution proved to be invariably so.

The above remarks apply to the effect of ammonium sulphide against Hop-mildew. In 1915 ammonium sulphide containing 0·13 % or 0·16 % sulphide-sulphur proved fungicidal against American Gooseberry-mildew growing in the open, without causing any injury to the leaves or tips of the shoots, or disfigurement to the berries.

Patches of mildew treated with ammonium sulphide of a fungicidal strength remain white and but little altered in appearance to the superficial view, except that the mycelium may be in places more or less collapsed and flocculent; it remains persistently sterile until it passes into a dying condition. In Exper. 5, where a solution containing 0·08 % sulphide-sulphur was used on Hop-plants, the mildewed apex of a shoot (with the mildew completely encircling the stem) was sprayed, as well as the leaves bearing mildew. From the 1st day after spraying to the 18th day (when the experiment was concluded) the mildew on the upper leaves and on the stem remained white, although rendered completely sterile by the fungicide.

In Exper. 9, where a solution containing 0·13 % sulphide-sulphur was used, Hop-leaves bearing mildew, and also the healthy apex of a shoot and two healthy leaves, were sprayed *on both sides* of the leaf and *all round the shoot*; no injury resulted to the leaves or shoot. In one experiment (No. 12) the solution containing 0·16 % sulphide-sulphur caused serious injury to the Hop-leaves.

The only instance we can find recorded of the use of ammonium sulphide as a fungicide is that mentioned by Bourcart[1], who states that Dufour used it against *Dematophora necatrix* with negative results.

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[1] Bourcart, E., *loc. cit.* p. 99.

VI. AMMONIUM SULPHIDE (COLOURLESS).

Ammonium Hydrosulphide.

Exper. 1. *Solution (pale yellow) containing* 1·65 % S.S. *and* 1 % *soft soap.*

1st day. The patches checked, with the mycelium apparently more or less collapsed in places; some short conidiophores visible.

3rd day. The mildew apparently regrowing and forming fresh conidiophores.

7th day. The majority of the patches on each leaf now with vigorous tufts of almost or quite powdery conidiophores.

Exper. 2. *Solution (colourless) containing* 3·36 % S.S. *and* 1 % *soft soap.*

1st day. Patches slightly checked; hundreds of young conidiophores visible.

4th day. The majority of the patches, although still somewhat checked, showing groups of numerous, long conidiophores; the mycelium not collapsed and flocculent. On two out of the four leaves injury apparent in the form of a small brown "scorched" spot.

5th day. Some of the patches subpowdery.

7th day. The majority of the patches now "powdery."

Exper. 3. *Solution (yellow) containing* 3·31 % S.S. *and* 1 % *soft soap.* (This was the same solution as used above in Exper. 2, after one day's exposure to air in a half-filled stoppered bottle.)

2nd day. Mildew greatly checked.

3rd day. All the patches greatly checked, mostly sterile, but weak conidiophores visible here and there in several places on each leaf.

4th day. Mildew white, but dormant and sterile.

6th day. Patches mostly sterile, but some bearing a few weak scattered conidiophores.

10th day. Most of the patches on two leaves now with abundant conidiophores and almost powdery; on the third leaf many patches dead, but some patches bearing a few conidiophores.

Ammonium Sulphide (colourless).

Exper. 1. *Solution (colourless) containing* 1·68 % S.S. *and* 1 % *soft soap.*

1st day. Patches slightly checked; hundreds of young conidiophores visible. Slight traces of injury to some leaves.

4th day. Mildew still checked, with the mycelial hyphae apparently more or less collapsed in places; numerous groups of tall conidiophores produced freely on some of the patches. Distinct injury in the form of brown (dead) patches of leaf-cells on two leaves; similar injury, but more slight, on the four other leaves.

5th day. A few patches now subpowdery, but the mildew on the whole still checked.

7th day. The majority of the patches now "powdery" or subpowdery.

Exper. 2. *Solution (pale yellow) containing* 1·68 % S.S. *and* 1 % *soft soap.*

1st day. Mildew checked; all the patches showing more or less collapsed mycelium.

3rd day. All the patches sterile.

7th day. Most of the patches still sterile with collapsed mycelium; on each leaf, however, a few patches showed scattered erect new conidiophores.

10th day. No further growth of the mildew, which was practically entirely dormant and sterile.

Exper. 3. *Solution (yellow) containing* 1·68 % S.S. *and* 1 % *soft soap.* (This was the same solution as used above in Exper. 1, after one day's exposure to air in a half-filled stoppered bottle.)

2nd day. Mildew much checked, with collapsed mycelium.

3rd day. Mildew mostly sterile, but here and there a few short conidiophores; slight "scorching" evident at the tip of two of the leaves.

4th day. All the patches apparently becoming sterile; no further injury to the leaves.

6th day. The mildew everywhere sterile.

10th day. The mildew sterile and apparently dead on the old leaves; on the younger leaves several patches bearing fairly vigorous tufts of conidiophores.

Hydrogen Sulphide

Exper. 1. *Solution containing* 0·056 % S.S. *and* 1 % *soft soap.*

3rd day. The mildew quite unaffected; all the patches "powdery."

5th day. All the patches as "powdery" as those on the control leaves—in some cases more so; no injury to the leaf

Summary of Observations. VI.

It will be seen from the foregoing experiments that the sulphur of hydrogen sulphide is without detrimental effect upon the mildew, although the concentration at which this was used is such as to be comparable only with our experiments with the weaker concentrations of yellow ammonium sulphide.

Ammonium hydrosulphide free from polysulphides is seen to be only slightly detrimental to the fungus and clearly is less efficacious as a fungicide than ammonium sulphide, also free from polysulphides, at the same concentration of sulphide-sulphur. At double the concentration, however, its behaviour towards the mildew does appear to approach that of the colourless ammonium sulphide.

When colourless solutions of ammonium hydrosulphide and of ammonium sulphide are allowed to stand exposed to atmospheric oxygen, they develop a yellow colour due to the formation of polysulphides in the solutions and it is observed that this change, although not altering appreciably the concentration of sulphide-sulphur, is attended by an increased fungicidal action of these solutions.

From these results it seems clear that the form in which sulphur functions as a fungicide in these solutions is that known as the polysulphide form.

VII. LIME-SULPHUR.

Hops. 1914.

Exper. 1. *Solution of* 1·005 *sp. gr.* (*containing* 0·7 % S.S.) *and* 0·125 % *saponin.*

1st day. Most of the patches well covered over by the deposit; a few patches showing the conidiophores uncovered or breaking through.

3rd day. As above.

5th day. Conidiophores still visible on some patches, but not apparently increasing

8th day. Many of the patches dead; the few alive very weak.

12th day. A very few conidiophores visible on a few patches.

Exper. 2. *Solution of* 1·005 *sp. gr.* (*containing* 0·7 % S.S.) *and* 0·25 % *saponin.*

1st day. Many of the patches showing the conidiophores uncovered by the deposit.

3rd day. Nearly all the patches showing the conidiophores matted together; on a few patches the conidiophores were erect and apparently vigorous.

5th day. As above.

8th day. Nearly all the patches apparently dying or dead; in a very few cases a minute group of conidiophores was visible.

12th day. Some patches still showing a few conidiophores.

Exper. 3. *Solution of* 1·005 *sp. gr.* (*containing* 0·7 % S.S.) *and* 0·25 % *saponin.*

1st day. The patches all well covered over, some showing the conidiophores still erect, some with conidiophores collapsed.

4th day. No new conidiophores formed; all the patches still well covered.

6th day. All the patches remaining dormant.

9th day. All the patches dead.

Exper. 4. *Solution of* 1·01 *sp. gr.* (*containing* 1·43 % S.S.).

1st day. The patches well covered, their conidiophores erect and covered over by the "pellicle" formed by the dry spray.

3rd day. Patches apparently quite dormant; the conidiophores visible, but no fresh ones formed.

5th day. Some of the patches beginning to die; the conidiophores beginning to collapse.

7th day. The mildew dead; the leaves now beginning to turn yellow.

Exper. 5. *Solution of* 1·01 *sp. gr.* (*containing* 1·43 % S.S.) *and* 0·25 % *saponin.*

1st day. The patches well covered over.

3rd day. All the patches quite dormant.

5th day. All the patches apparently dying or dead.

7th day. All the patches dead; no injury to the leaf.

Exper 6. *Solution of* 1·01 *sp. gr.* (*containing* 1·43 % S.S.) *and* 0·25 % *saponin.*

1st day. All the patches well covered over; some showing conidiophores still erect, some with conidiophores collapsed.

4th day. The conidiophores still visible on the patches, but no fresh ones being produced; the mycelium remaining dormant.

6th day. All the patches quite dormant.

10th day. Two patches showing a very few weak scattered conidiophores; the remaining patches all dead.

Exper. 7. *Solution of* 1·01 *sp. gr.* (*containing* 1·43 % S.S.) *and* 0·25 % *saponin.*

1st day. Most of the patches well covered over with the deposit; some of the patches showing the conidiophores breaking through the deposit.

3rd day. All the patches showing matted or collapsed conidiophores.

5th day. All the patches still dormant.

8th day. All the patches dead.

Exper. 8. *Solution of* 1·01 *sp. gr.* (*containing* 1·43 % S.S.) *and* 0·125 % *saponin.*

1st day. All the patches well covered over with the deposit.

3rd day. As above.

5th day. Conidiophores, more or less collapsed, visible on most of the patches; no fresh formation of conidiophores.

8th day. All the patches dead; the mycelium and conidiophores shrivelled up.

Summary of Observations. VII.

As it is impossible to use soap with lime-sulphur solutions, it was decided to substitute saponin so as to make the experiments comparable as far as possible with those where soap was used. At 1·005 sp. gr. the lime-sulphur solution was either quite, or was very nearly, fungicidal; at 1·01 sp. gr. it was almost invariably so.

These results are corroborative of the results previously obtained by one of us[1] in certain experiments in which lime-sulphur at 1·01 sp. gr. proved completely fungicidal against *S. Humuli*, killing the growing fungus and preventing infection of the leaf by conidia.

VIII. IRON SULPHIDE.

Hops. 1914.

Exper. 1. *Solution containing* 0·2 % *iron sulphide.*

1st day. Mildew only slightly checked; conidiophores still mostly erect.

3rd day. Some of the patches (perhaps those where the spray did not permeate) now almost "powdery"; others still checked.

5th day. The majority of the patches now with numerous erect conidiophores.

7th day. A considerable number of the patches now "powdery."

Exper. 2. *Solution containing* 0·2 % *iron sulphide and* 0·25 % *saponin.*

1st day. Mildew only slightly checked; conidiophores still mostly erect and vigorous.

3rd day. Some of the patches nearly "powdery."

5th day. Most of the patches now with very numerous erect conidiophores.

7th day. All the patches now "powdery" or nearly so.

Exper. 3. *Solution containing* 0·2 % *iron sulphide and* 1 % *soft soap.*

1st day. Mildew checked.

3rd day. All the patches still more or less checked.

5th day. The patches now showing very numerous erect conidiophores.

7th day. All the patches now "powdery" or nearly so.

Exper. 4. *Solution containing* 0·3 % *iron sulphide and* 0·5 % *soft soap.*

1st day. Mildew much checked; a few scattered short conidiophores visible.

3rd day. All the patches quite dormant and apparently dying.

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[1] Salmon, E. S., "Report on Economic Mycology" (*Journ. S.-E. Agric. College*, XIX. p. 345 (1910)).

5th day. As above.

8th day. The majority of the patches dead on all the three leaves; on each of the two lower leaves three patches only were alive—one bearing a minute group of conidiophores, the remaining two dormant and sterile; on the upper leaf several patches bore a group of conidiophores.

12th day. The mildew still feebly alive at a few spots on the two lower leaves; on the upper leaf several of the patches showed a more or less vigorous growth and bore conidiophores.

Exper. 5. *Solution containing* 0·3 % *iron sulphide and* 1 % *soft soap.*

1st day. Mildew checked.

3rd day. All the patches quite sterile and dormant.

5th day. As above.

8th day. Patches all apparently dying.

12th day. As above.

Exper. 6. *Solution containing* 0·3 % *iron sulphide and* 1 % *soft soap.*

1st day. Mildew greatly checked, many of the patches apparently killed.

2nd day. Nearly all the patches dead; a few patches near the edge of the leaf developing a few conidiophores.

5th day. As above.

7th day. The few patches noted above remaining alive and bearing small isolated groups of conidiophores.

11th day. The groups of conidiophores at the few patches more numerous.

Exper. 7. *Solution containing* 0·6 % *iron sulphide and* 0·5 % *soft soap.*

1st day. The mildew greatly checked or killed.

3rd day. All the patches quite dormant and apparently dying.

5th day. All the patches dead.

Exper. 8. *Solution containing* 0·6 % *iron sulphide and* 1 % *soft soap.*

1st day. All the patches well covered over with the brown deposit, and showing only collapsed conidiophores.

4th day. All the patches dead; leaf uninjured.

Exper. 9. *Solution containing* 0·6 % *iron sulphide and* 1 % *soft soap.*

1st day. Mildew very greatly checked; most of the patches apparently killed.

3rd day. Nearly all the patches dead; only one or two on each leaf showing a few of the conidiophores remaining.

5th day. All the patches apparently dead.

12th day. All the patches dead.

Exper. 10. *Solution containing* 0·45 % *iron sulphide and* 1 % *soft soap.*

1st day. All the patches of mildew completely covered by the spray and apparently killed; no injury to the leaves apparent.

4th day. All the patches of mildew dead; the youngest sprayed leaf showing injury at the tip and margin.

6th day. The youngest leaf now severely injured; the remaining leaves unaffected.

9th day. No further injury to the leaves.

Exper. 11. *Solution containing* 0·9 % *iron sulphide and* 1 % *soft soap.*

1st day. All the patches of mildew completely covered by the spray and apparently killed; the youngest of the sprayed leaves injured and shrivelled.

4th day. All the patches of mildew dead; injury apparent to the tip and margins of another leaf—the remaining leaves (four) not injured

9th day. No further injury to the leaves.

Summary of Observations. VIII.

The iron sulphide solution dries at once to a rusty-brown deposit which covers over uniformly the patches of mildew[1]. A 0·3 % solution, with a 1 % solution of soft soap, is almost satisfactory as a fungicide; a 0·6 % solution, with either a 0·5 % or 1 % solution of soft soap, is invariably fungicidal. No injury was caused to the foliage. In the above experiments the iron sulphide was made by the method (i) (described at p. 480) under which it was possible for a small quantity of ammonium sulphide to be present. In two experiments (carried out in 1915) the iron sulphide was made on the method (ii) (described at p. 480) under which no ammonium sulphide was present. In the first experiment (No. 10) a 0·45 % solution of iron sulphide was used; it proved completely fungicidal by the 4th day. Severe injury was caused to the youngest Hop-leaf, the others remaining uninjured. In the second experiment (No. 11) a 0·9 % solution was used; the mildew was killed immediately; two out of the six leaves were injured at the tips and margins.

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[1] **If used on Gooseberries it would be inferior to the yellow ammonium sulphide solution because it would disfigure the berries; it would have the advantage, however, over lime-sulphur in that the markings on the sprayed berries would not be liable to be mistaken for spots of mildew by incompetent inspectors at the market.**

THEORETICAL CONSIDERATIONS.

Although a considerable literature is to be found dealing with alkaline sprays—particularly those known as "lime-sulphur" and "liver-of-sulphur"-- there appears to be little of a definite character known indicating which of the several constituents of these mixtures possess the fungicidal properties associated with these spray-fluids.

A consideration of the results obtained in our experiments makes it evident that certain of the views referred to above (p. 475) are erroneously founded, although sufficient data have not yet been obtained on which to base a full discussion of the problem.

It has been claimed that solutions of "liver-of-sulphur" are fungicidal mainly by reason of the free alkali they contain initially or by reason of that which may become available subsequent to spraying. That this explanation does not hold good appears clear from the following evidence based on results obtained in our experiments with solutions of caustic soda and of a solution of "liver-of-sulphur" which is fungicidal. Accepting a 1 % solution of the sample of "liver-of-sulphur" used in our experiments as being definitely fungicidal, calculation shows that this cannot contain more free alkali than the equivalent of 0·4 % NaOH, whereas a solution containing 0·5 % NaOH proved to be not fungicidal. Thus it seems clear that a concentration of "liver-of-sulphur" which is entirely fungicidal will not contain at any time a sufficient concentration of free alkali to destroy the mildew.

Equally clear evidence of the inefficacy of free alkali has been found in the case of yellow ammonium sulphide. Solutions of this compound which cannot, by reason of their mode of preparation, contain as much as 0·4 % total ammonia are found to be fatal to the mildew (*vide* Yellow Ammonium Sulphide, Expers. 9, 10 and 11); whereas our experiments with solutions of ammonia show that this alkali up to 2 % concentration is not detrimental to the fungus.

The negative results obtained in our experiments with solutions of sodium carbonate also give the same evidence that the weak alkaline nature of the spray-fluid is not responsible for fungicidal action.

In view of the fact that the ammonium sulphide solution used—which proved so efficacious—contained no sulphate and only indeterminably small quantities of thiosulphate and of sulphite, this discounts very largely the suggestion that any of these substances are responsible for the fungicidal action observed. It was not surprising therefore to find that sodium thiosulphate exhibited no fungicidal properties.

A comparison of the results of our experiments makes it clear that the proportion of "sulphide-sulphur" present is no index of the efficacy of a spray-fluid[1]. It will be seen that a solution of yellow ammonium sulphide containing 0·13 % sulphide-sulphur is invariably fungicidal, whereas a solution containing 0·13 % sulphide-sulphur in the case of "liver-of-sulphur" has no fungicidal value—the concentration at which the latter substance becomes fungicidal is 0·34 % sulphide-sulphur.

Further, solutions containing as much as 1·6 % and 3·3 % sulphide-sulphur in the case of a colourless solution of ammonium hydrosulphide and a colourless solution of ammonium sulphide respectively failed to do more than check the fungus temporarily.

It seems evident, therefore, that the soluble polysulphides present are the substances of fungicidal value. The question which then arises is whether the property of killing the fungus is due to the direct action of the polysulphides themselves or whether it is due to the sulphur which is deposited when these compounds decompose.

With the object of gaining information on this point determinations have been made of the amount of sulphur which is deposited from a solution of colourless ammonium hydrosulphide and from a solution of yellow ammonium sulphide when exposed to air under conditions similar to those obtaining when these substances are used as spray-fluids.

Definite quantities of these solutions were absorbed on tared filter-papers and allowed to dry in the air alongside similar filter-papers which served as controls and as counterpoises in weighing. The solution of ammonium hydrosulphide used contained 5·93 % of "sulphide-sulphur" (somewhat less than the stock solution used in Exper. 2, i.e. 6·7 %) and it was found to deposit 0·356 grm. per 100 c.c. of solution. The solution of yellow ammonium sulphide contained 2·19 % of "sulphide-sulphur" (the stock solution from which the spray-fluids have been prepared) and, in this case, the deposit of sulphur amounted to 2·56 grms. per 100 c.c. of solution.

From these estimations it is calculated that when the above-mentioned solutions are diluted in the manner followed when preparing such solutions for spraying purposes 100 c.c. of the ammonium hydrosulphide spray-fluid (containing in this case 2·96 % sulphide-sulphur:

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[1] In an article in the *Journ. Board of Agric.* 1914, vol. XXI. p. 236, giving the analyses of various commercial samples of "liver-of-sulphur," the assumption has been made that the fungicidal value of any sample is determined by its sulphide-sulphur content.

compare Expers. 1 and 2) would deposit 0·178 grm. of sulphur; and 100 c.c. of the yellow ammonium sulphide spray-fluid (containing 0·13 % sulphide-sulphur: compare Expers. 9 and 10, p. 494) would deposit 0·160 grm. of sulphur.

This experiment was repeated three times and closely similar results were obtained in each case showing that the amount of sulphur actually deposited from these two kinds of alkaline sulphide solutions is not widely different. It would seem therefore that a larger deposition of sulphur occurred in the case of our Expers. 1 and 2 with ammonium hydrosulphide than in Expers. 9 and 10, p. 494 with yellow ammonium sulphide. In view of the considerable interest attaching to this part of the subject, it is proposed to repeat and extend these experiments during the coming season.

From the results so far obtained the conclusion is reached that yellow ammonium sulphide is valuable as a fungicide because of the polysulphides contained in solution and that these probably act as such and not by virtue of sulphur which is deposited. It is thought that when a solution of ammonium hydrosulphide is allowed to evaporate in the air the oxidation which takes place does not largely lead to the formation of polysulphides but mainly to the direct deposition of sulphur.

GENERAL SUMMARY AND CONCLUSIONS.

1. Solutions of such substances as "liver-of-sulphur" and ammonium sulphide when used against the "powdery mildews" (*Erysiphaceae*) in the actively-growing conidial stage require the addition of some substance such as soft soap in order to increase their wetting properties and so secure complete fungicidal action.

2. Solutions of "liver-of-sulphur" of the strength recommended by authors generally (0·2 % to 0·4 %) for use against the "powdery mildews" are not fungicidal against the growing mycelium. When the concentration is increased to 0·6 % or 0·8 % this substance begins to be fungicidal.

3. A solution of yellow ammonium sulphide has proved to be completely efficacious against the Hop-mildew (in the greenhouse) and the American Gooseberry-mildew (in the open). The use of this material has the distinct advantage that, unlike lime-sulphur, it leaves no visible deposit and does not therefore disfigure the fruit. Solutions of definite fungicidal strength have caused no "scorching" injury to the foliage of the Hop or Gooseberry.

4. Iron sulphide, which has been favourably reported upon in field experiments, proved on close observation to have, at a concentration of 0·6 %, a remarkably quick fungicidal action on the Hop-mildew. When made by a method which leaves a trace of ammonium sulphide present it has proved to be quite harmless to foliage and is in a condition which enables it to be applied as a fine spray.

5. The presence of free alkali in solution is not the determining factor in the fungicidal value of the alkaline sulphide solutions used.

6. The proportion of sulphide-sulphur present is no index of the fungicidal value of solutions of alkaline sulphides.

7. It appears that the polysulphides contained in a solution of yellow ammonium sulphide act fungicidally as such and not by virtue of the sulphur which is deposited when these compounds decompose.

(*Received December 27th*, 1915.)

SOME OBSERVATIONS ON THE FLORA AND FAUNA OF FLOODED FENLAND.

By F. R. PETHERBRIDGE, M.A.

(*School of Agriculture, Cambridge University.*)

On January 3rd, 1915, the Little Ouse burst its right bank not far from the Feltwell 2nd District Pumping Station, and as a result thousands of acres of fenland became flooded. The portion of the flooded land on which the following observations were made is a part of Southery fen situated near Southery, Norfolk. It lies between Peckett's Farm and Turf Fen Farm, and belongs to the Norfolk County Council. This land is some distance from the place where the burst occurred, and as a consequence there was no deposit of silt. The height of the flood here was about nine feet. The water was gradually pumped off and the land cleared by September, 1915.

Heavy rains kept patches of the land very wet until December, 1915, when the following observations were made. After the water had all been cleared from the land, the most striking feature was that a large portion of the land was completely covered by the alga *Cladophora flavescens*, known locally as "blanket" or "carpet weed." This mat of the dead and dying alga was about an inch thick and could be lifted up from the soil in the same way as a carpet can be lifted from the floor (Pl. V, fig. 1). When dry this carpet made ploughing very tedious, as the plough frequently became clogged. On some of the fields it was raked into heaps and burnt before ploughing.

In the damper spots small patches of *Vaucheria terrestris* and *Enteromorpha intestinalis* were found together with the *Cladophora*.

Another noticeable feature was the presence of *Polygonum amphibium* in great abundance. Some fields were so thick that from a distance the reddish brown colour of these plants only was noticeable, the blanket weed being underneath.

Polygonum amphibium usually floats and reaches the surface of the water in which it grows, so from the length of these plants (many of which were over nine feet long) it was possible to tell the depth of the water on the different fields. Most of the plants were carrying a good supply of seed so that it may prove troublesome during the next few years. It caused more hindrance to ploughing than the "blanket weed."

Chara hispida was present in great abundance for a distance of about sixty feet on each side of the dykes. Sometimes it was abundant on one side of the dyke and not on the other. On the rest of the field only small patches could be found (Pl. V, fig. 2).

Another smaller species of *Chara* was found but not very frequently.

As the remains of the *Charas* are almost entirely composed of calcium carbonate, they should prove of considerable value in neutralizing the acidity of the soil, and it will be interesting to note in those fields which receive no dressings of lime, chalk, or similar materials, if the crops grow better where the deposits of *Chara* were thickest, viz. near the dykes.

In places, *Alisma plantago*, the water plantain, was prevalent and had set seed (Pl. VI, fig. 3).

Occasional plants of *Typha latifolia* were met with, but these were cut down before the land was ploughed.

On the dyke sides *Arundo phragmites* was growing luxuriantly.

The following is a list of the plants found on the arable land:

Polygonum amphibium
Alisma plantago
Typha latifolia
Mentha aquatica
Agropyrum repens
Agrostis alba
Cnicus arvensis
Callitriche verna var.

Polygonum aviculare
Veronica beccabunga
Ranunculus aquatilis var.
Oenanthe fluviatilis
Lythrum salicaria
Senebiera coronopus
Rumex hydrolapathum
Glyceria aquatica (near the dykes)

Grass Land. Two pastures were examined, situated respectively near the farm buildings of Peckett's Farm and Turf Fen Farm. On both of these all the useful grasses were killed. Both were thickly covered with "blanket weed," and occasional specimens of *Polygonum amphibium* were found. *Agropyrum repens* was present in abundance, and occasional specimens of *Agrostis alba* were found.

At Turf Fen Farm, *Potentilla anserina* was fairly abundant, as was also the moss *Amblystegium riparium.* Both of these pastures are destined to be ploughed up.

As a result of the flood, many of the stacks were moved from the stackyards, and some floated considerable distances. After the water had subsided, the stacks became covered with plants up to the water level.

The following were found growing on old straw stacks:

Matricaria inodora
Stellaria media
Viola tricolor
Galeopsis versicolor
Chenopodium album
Galium aparine
,, *uliginosum*
Lapsana communis
Stachys palustris
Polygonum aviculare
Plantago major
Agrostis alba
Mentha aquatica
Senecia vulgaris

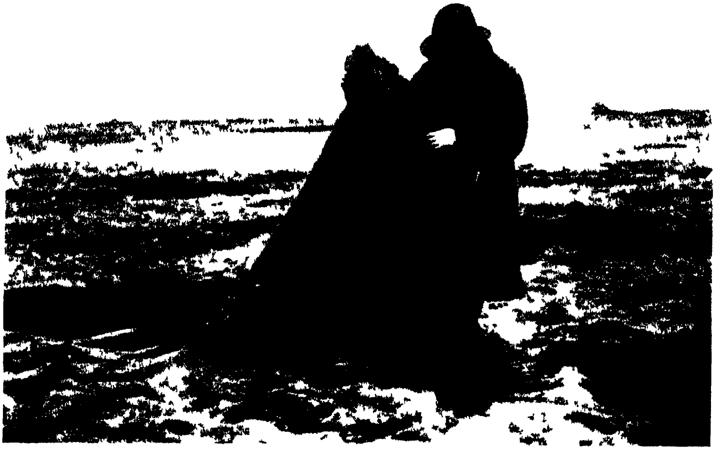
The flora of the farmyard was very varied, many of the plants probably originating in the straw stacks.

Polygonum amphibium
,, *persicaria*
,, *fagopyrum* (buckwheat)
,, *aviculare*
Sinapis arvensis
Alisma plantago
Galeopsis versicolor
Chenopodium album
,, *urbicum*
Urtica urens
,, *dioica*
Lapsana communis
Mentha aquatica
Stellaria media
Carduus nutans
Lychnis Githago
Plantago major
Stachys palustris
Capsella bursa pastoris
Ranunculus repens
,, *sceleratus*
Nasturtium officinale
Barbarea vulgaris
Rumex obtusifolius
Poa annua
,, *trivialis*
Dactylis glomerata
Agropyrum repens
Agrostis alba

In the gardens all flowers, vegetables, box hedges and gooseberry bushes were killed with the exception of horse radish. All the willow trees along the sides of the dykes formed dense masses of adventitious roots above the soil level and below the flood water level (see Pl. VI, fig. 4). The soil reacted slightly acid to litmus and nitrifying organisms were found to be present.

FAUNA.

Snails. It was thought probable that some of the flooded land might be infested with *Limnaea truncatula*—the snail which carries the Liver-fluke of sheep—but none were found.

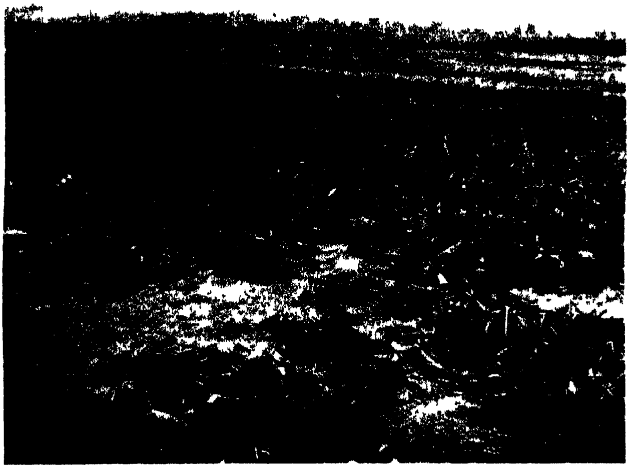


Cladophora flavescens ("blanket" or "carpet weed") and *Polygonum amphibium*

Fig. 1

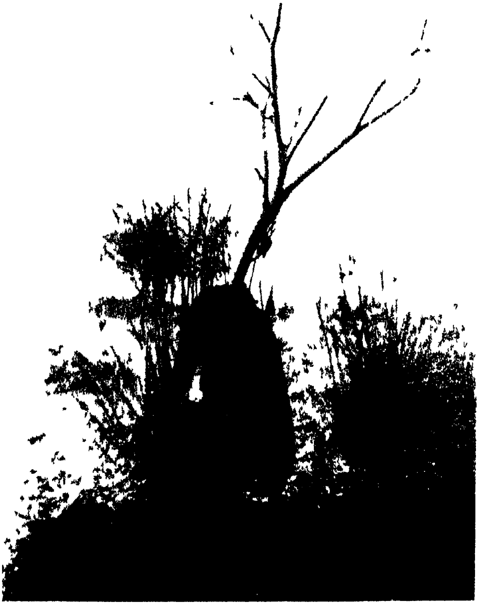
The zone of *Chara hispida* spreading from the dyke on the right, with scattered plants of *Alisma plantago*

Fig. 2



Area densely covered with *Alisma plantago* with a patch of *Cladophora flavescens* in the foreground

Fig. 3



Salix alba showing the adventitious roots formed below the flood water level.

Fig. 4

A large number of empty shells and a few living specimens of the following were found:

Limnaea pereger
„ *stagnalis*
Planorbis vortex
Planorbis umbellicatus
„ *corneus*
Bythinia tentaculata

Eelworms. The presence of eelworms was tested for by placing small pieces of lean meat and mangold on the surface of the soil in the laboratory and examining at intervals. No eelworms were found.

Earthworms. A few earthworms were found in the stackyards, but none in the fields.

Insects. Large numbers of bloodworms, the larvae of various species of *Chironomus*, were found. These were present in great abundance all over the area.

The only other insect found in the fields was a larva of a hover fly.

(*Received January 25th,* 1916.)

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